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Vol. 1. No. 2. FEBRUARY 1948

JOURNAL OF CLINICAL PATHOLOGY

EDITED FOR

THE ASSOCIATION OF CLINICAL PATHOLOGISTS

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LONDON

BRITISH MEDICAL ASSOCIATION

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J. clin. Path. (1948), 1, 57.

IRON METABOLISM

BY

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(RECEIVED FOR PUBLICATION, JANUARY 5, 1948)

Ten years ago physiologists still taught that the iron metabolism of the body was regulated by a balance between absorption from the stomach and small intestine and excretion by the colon. McCance and Widdowson (1937a) first challenged this theory, arguing from facts already known that there was no evidence of excretion of iron by the body except in very small amounts, and that therefore the iron balance must be maintained by a regulation of iron absorption. In confirmation of this view McCance and Widdowson (1938) showed that when normal subjects were given iron by mouth they excreted an equal amount in the faeces, whereas when iron was given by intravenous injection the whole was retained and none excreted. The logic of this hypothesis compelled its immediate acceptance, but it could not be finally proved until a dose of iron could be "tagged" by the radio-active element so that its whole course through the body could be followed.

The principles of experiments with radio-active elements are now familiar. A minute proportion of the atoms is made radio-active by artificial means. The whereabouts and number of such atoms can be traced by measuring their radio-activity, so that they can be used to "tag" a much greater number of normal atoms, from which they do not differ either chemically or biologically. Radio-active iron is unfortunately one of the more difficult elements to work with, for its radiation is very soft and is readily absorbed by other metals. The radio-active iron must therefore be isolated in a very pure state before it can be measured, and even the metallic iron must be in a very thin layer to avoid errors from self-absorption of radiation.

Physiological Experiments with Radio-active Iron

Radio-active iron appears in the plasma soon after it is fed to a dog and reaches its maximum concentration in four to eight hours. It is, however, quickly taken up by the storage depots, and after a few hours none can be detected in the plasma (Hahn and others, 1939). The iron in the storage depots is very mobile, for radio-active iron can be detected in the red cells within four hours of its administration by mouth. The amount of radio-active iron in the circulating red cells thereafter increases progressively to a maximum which is reached in four to seven days in anaemic dogs (Hahn and others, 1940). The level subsequently remains constant. It can therefore be presumed that all the radio-active iron absorbed has been used in haemoglobin formation, and that none remains in the storage depots.

Much work with radio-active iron has been based on this assumption that iron utilization is a measure of absorption. The supposition is certainly justified when there is iron-deficiency anaemia, in that Dubach and others (1946) have shown that even injected radio-active iron is completely utilized in six to nine days by dogs and men in this condition. In normal animals, however, there is a greater probability that some radio-active iron absorbed from the intestine may be diverted to storage depots and not appear in the circulating red cells during the course of the experiment, but it is unlikely that errors from this source have been great enough to invalidate the major conclusions from the experiments to be described below.

Iron Absorption

Iron is absorbed only in the stomach and small intestine, and not at all in the colon. When a dog is given radio-active iron by mouth the highest plasma level is reached in four to eight hours, and since this is the time when the meal is in the small intestine it is here that the maximum absorption takes place. After eighteen to twenty-four hours, when the whole meal has passed into the colon, no further absorption of radio-active iron occurs (Hahn and others, 1939a). It has also been shown that radio-active iron is absorbed into the blood stream from gastric, duodenal, and jejunal pouches in the dog (Hahn and others, 1943).

A man's daily loss of iron from the body is of the order of only 1 mg., so there must evidently

be some selective control of iron absorption—otherwise haemochromatosis would be a common complication of old age. The gap between an adequate supply and a deficiency of iron is, however, very narrow, and many factors may inhibit the absorption of iron which the body needs to maintain the haemoglobin level.

The control of iron absorption.—The body absorbs iron according to its needs, in health only enough to replace the small daily loss, but in iron deficiency as much as is offered and physiologically available. It is a common experience that a patient being treated for iron-deficiency anaemia may regenerate haemoglobin at a rate which demands the absorption of 50 mg. of iron a day, whereas McCance and Widdowson (1938) showed that when extra iron is given to a normal individual he excretes it all in his faeces. Hahn and others (1940) showed very clearly the difference in radio-active iron absorption by normal and anaemic dogs. Whereas a normal dog would absorb only very little of a test-dose given by mouth, and a plethoric dog would absorb practically none, dogs suffering from chronic iron-deficiency anaemia might utilize more than half of a small test-dose for haemoglobin production.

However, the degree of iron absorption is not determined by the haemoglobin level *per se*. Thus the intestinal iron absorption of a normal dog is not immediately increased if it is made suddenly anaemic by bleeding, but after a week, when the iron reserves are depleted by haematopoiesis, a test-dose of radio-active iron is vigorously absorbed. Nor will prolonged anoxia increase iron absorption.

Studies in man have given very similar results (Balfour and others, 1942). Normal individuals absorb very little of a test-dose of radio-active iron given by mouth, but absorption is ten times as great in patients with chronic iron-deficiency anaemia. In polycythaemia, on the other hand, only a trace of radio-active iron is absorbed, in spite of the excessive formation of red cells and haemoglobin.

Pregnant women, whether they are anaemic or not, absorb between two and ten times as much of a test-dose of radio-active iron as do normal individuals (Balfour and others, 1942). Here the degree of iron absorption evidently depends upon the demands of the foetus rather than upon any state of anaemia in the mother.

The iron-absorbing powers of the intestine are readily exhausted. Thus a dose of an iron salt given by mouth will diminish the absorption of radio-active iron administered one or two hours later. The inhibition is not due to the elevation of the plasma iron level by the first dose, for an intravenous injection of iron does not reduce the absorption of radio-active iron given by mouth (Hahn and others, 1943).

Hahn has explained the above facts by postulating that the cells of the intestinal mucosa contain a protein receptor which can receive iron ions from the intestinal contents and yield them to the plasma. Thus the absorption of iron from the intestine depends on the presence of free places in the receptor, and the rate of removal of iron from the intestinal cells into the blood is determined by the level of the plasma iron and its equilibrium with the tissue reserves. The reverse process, a transfer of iron from the plasma to the intestinal cells, is not possible because the plasma iron is attached to a plasma protein, probably globulin, and cannot be yielded to the intestinal receptor. The intestinal mucosa is very readily saturated with iron—within two hours if enough iron is available in the food—but desaturation into the plasma is not complete for some days

Granick (1946) has recently brought forward evidence that the iron receptor in the intestinal mucosa is apoferritin, the protein which binds the greater part of the tissue-storage iron *(vide infra)*. Iron combines with apoferritin to form ferritin, and the amount of this compound in the intestinal mucosa increases after feeding with iron, and falls again after several days. There is evidently some local mechanism for the formation or accumulation of apoferritin in the intestinal mucosa, for the total amount of ferritin + apoferritin increases in response to iron feeding.

Very little radio-active iron appears in the red cells of patients with pernicious anaemia when they are given a "tagged" iron salt by mouth. This finding has been interpreted as meaning that the patients, in spite of their severe anaemia, absorb no more iron than do normal people because their iron stores are not depleted. Dubach and others (1946), however, have cogently argued another interpretation. They point out that the blood-level of radio-active iron after a week or so measures only the iron utilized in haemoglobin synthesis, and that much more iron might have been absorbed and stored unused. They have in fact shown this to be the case in a man who had pernicious anaemia without any iron deficiency. In this patient only about 1 per cent of a test-dose of 57 mg. of radio-active iron appeared in the circulating haemoglobin until liver treatment was started nine days later. Then, as the haemoglobin level rose the proportion of the test-dose of radio-active iron in the blood likewise increased

until more than 20 per cent of the administered dose was found in the circulating haemoglobin. Thus the initial low level of circulating radio-active iron was a sign not of small absorption but of deficient haemoglobin synthesis and iron utilization. Dubach and others (1946) have made similar criticisms of the common statement that iron absorption is not increased in haemolytic anaemias.

The theory that iron metabolism is regulated by selective absorption from the stomach and intestine and not by excretion will doubtless demand many similar modifications in detail, but its basic truth still rests on the experiments and reasoning of McCance and Widdowson (1937a, b, 1938, 1943).

Iron valency and absorption.—It is the universal experience of clinicians that in the treatment of iron-deficiency anaemia ferrous salts are effective in about half the dose of ferric salts. Table I (from Witts, 1936) shows the iron content of the average daily doses of ferrous and ferric

TABLE I

IRON CONTENT AND UTILIZATION OF THE AVERAGE EFFECTIVE DOSE OF VARIOUS THERAPEUTIC PREPARATIONS (WITTS, 1936)

Preparation	Iron content of average dose	Per cent utilization
Ferrous chloride ..	100–200 mg.	12.5–25
Ferrous sulphate ..	180 mg.	14
Ferrous carbonate (Blaud's pill)	300–400 mg.	6–8
Ferric chloride ..	400 mg.	6
Ferric citrate ..	400 mg.	6
Iron and ammonium citrate	800–1,600 mg.	1.5–3

salts necessary to raise the total blood iron by 24 mg. per day, equivalent to a haemoglobin increase of about 0.14 g. per 100 ml. It is evident that ferrous salts are absorbed and utilized at least twice as efficiently as ferric salts.

Moore and others (1939) showed that serum iron absorption curves after the administration of iron salts were higher with ferrous than with ferric salts. The difference has been more accurately demonstrated by giving the same subjects successive doses of radio-active ferrous and ferric salts. Table II summarizes some of the experiments of Moore and others (1944). Normal men utilized 1.5 to 10 times as much ferrous as ferric salt, and in hypochromic anaemia the ratio was 2 to 15. Likewise Hahn and others (1945) showed that patients with hypochromic anaemia utilized 2 to 6 times as much ferrous as ferric salt when given successive test-doses of radio-active iron. Iron utilization can be taken as a measure of iron absorption in iron-deficient subjects if not in normal individuals, so that the superior absorption of ferrous ions is clearly demonstrated.

TABLE II

UTILIZATION OF FERROUS AND FERRIC RADIO-ACTIVE IRON SALTS BY NORMAL MEN AND IN HYPOCHROMIC ANAEMIA (MOORE AND OTHERS, 1944)

Form of iron	Dose given	Normal men		Hypochromic anaemia	
		No. of cases	Per cent utilization	No. of cases	Per cent utilization
Ferrous	4 mg.	4	7	2	12.5
Ferric	4 mg.	4	2.5	2	4
Ferrous	2 mg.	3	7.5	2	27.5
Ferric	2 mg.	3	2	2	4
Ferrous	1 mg.	2	9	1	39.5
Ferric	1 mg.	2	1.5	1	17

The same rule does not apply to all animals. Thus rats absorb ferric salts as well as ferrous (Austoni and Greenberg, 1940), as do many dogs, although the majority of both normal dogs (Moore and others, 1944) and anaemic dogs (Hahn and others, 1945) absorb a greater proportion of bivalent radio-active iron salts.

Moore and others (1944) offered three explanations for the superior absorption of ferrous salts. It is possible that only ferrous salts are capable of absorption, or that ferrous salts are much more readily absorbed than ferric, or that the ferric ions more readily form insoluble compounds in the intestine.

Relation of dosage to iron absorption.—Clinical experience has shown that the response of iron deficiency anaemia to medication depends upon the dose of iron given, and that there is often a minimum "threshold" dose below which treatment is ineffective. It is not clear to what extent this varying response depends upon the proportion of iron absorbed from the intestine, and to what extent upon iron utilization for haemoglobin synthesis. Most experiments with radio-active iron have shown that the higher the test-dose the less is the proportion which appears in the red cells, even though the total utilization may increase (see, for example, Table II). This has commonly been taken to mean that the proportion of iron

absorbed decreases with increasing dose, but it may be rather that with a larger dose a less amount of the iron absorbed is utilized. Brock and Hunter (1937), for example, showed by balance experiments that when large doses of iron were administered by mouth, twice as much iron might be retained in the body as was utilized for haemoglobin synthesis.

Dietary sources of iron.—It is easy to estimate the total iron content of a foodstuff or diet, but it is not usually possible to say what proportion of the iron is potentially available for haemoglobin synthesis. It is now known that only ionic iron can be absorbed from the intestine, but there is no rule for determining how much of the food iron can be freed in the ionic state by digestion. Iron also very readily forms insoluble compounds with various constituents of the diet, especially in a medium which is not strongly acid.

It has been amply demonstrated by both clinical and experimental studies that ionic iron is readily absorbed by the stomach and small intestine, but there is no evidence that any pre-formed iron compound can be absorbed as such and used for haemoglobin synthesis. The dietary iron is in two forms—inorganic, which readily passes into solution, and organic, chiefly porphyrin compounds which must be broken down before ionic iron is liberated. The relative proportions of the two can be distinguished by the α–dipyridyl reaction for ionizable iron, and at one time many estimates were made of the "availability" of iron in food by this reaction. Such estimates, however, bear only a very rough relation to the truly physiological availability of dietary iron. Both ferrous and ferric chloride, for example, are "100 per cent available" according to the α dipyridyl reaction but neither is completely absorbed from the intestine even in severe iron-deficiency anaemia, and at least twice as much of the ferrous as of the ferric salt is assimilated.

Some of the "non-available" food iron which is not estimated by the α–dipyridyl reaction may also be broken down and absorbed. Thus Black and Powell (1942) found that 10 to 20 per cent of haemoglobin iron was absorbed when a litre of citrated blood was given by duodenal tube. They considered bacterial decomposition in the intestine to be important in making this iron available.

The chemical nature of iron compounds in foodstuffs is, however, probably less important for their absorption than other even less easily defined factors. Conditions in the small intestine, except in its upper part, are very unfavourable to the absorption of iron. Iron salts are scarcely dissociated in solution at a *p*H above 5.0, so that few ferrous, and even fewer ferric, ions are available for absorption except in distinctly acid medium. It is not surprising, therefore, that iron-deficiency anaemia is commonly associated with gastric achlorhydria.

Iron phosphates are practically insoluble, and the ratio of phosphorus to iron in ordinary diets often exceeds 50, so that there is abundant opportunity for the formation of iron phosphates in the intestine. Phosphates may inhibit iron absorption even when no precipitate is formed, for Brock and Taylor (1934) found that the dialysis of iron salts across cellophane membranes was greatly decreased in the presence of neutral or alkaline phosphates.

Iron forms undissociated compounds with a number of amino-acids and phosphorus-containing substances such as nucleic acid, and McCance and others (1943) have emphasized that an excess of phytic acid in the diet may lead to the deviation of much iron as insoluble phytate.

An excess of calcium in the diets of animals usually inhibits iron absorption so that a mild iron-deficiency anaemia develops, but under some circumstances an excess of calcium favours iron absorption. Thus the excess phytic acid of wholemeal bread may be neutralized by calcium (McCance and others, 1943), and when the phosphate content of the diet is high a high level of calcium diminishes the formation of insoluble iron phosphates.

The vitamin content of a diet may have a direct influence on iron absorption. Ascorbic acid reduces ferric compounds to ferrous *in vivo* as well as *in vitro*, and thus facilitates absorption (Moore and others, 1939). Extra vitamin D increases haemoglobin formation and iron storage in young rats (Fuhr and Steenbock, 1943).

It is evidently difficult to decide whether a given diet will supply enough iron to maintain the haemoglobin level, and in practice the question is decided by experience rather than by any scientific knowledge of the physiological availability of iron in various foods. An ordinary mixed diet of the type eaten in England will provide enough iron for a normal man if its total iron content exceeds 5 mg. daily, and a total of 15 mg. daily will suffice a normal woman or growing child. The average English diet to-day probably gives 15–20 mg. of iron daily, an amount which in theory is rather more than adequate for women and children, and generous for men. The margin for women is small, however, and it requires relatively little disturbance of iron absorption from such causes as

gastric achlorhydria, or relatively little increased iron loss from menorrhagia or frequent child-bearing, to produce an iron deficiency.

The value of foodstuffs as iron sources is similarly a matter of empirical rather than scientific knowledge. Liver, eggs, dried fruits, chocolate, sardines, and green vegetables are all good sources, but nowadays are not freely available. Oatmeal, peas, beans, and whole-meal bread are also good sources, but white bread contains less than one-third of the original iron of the grain. Such staple foods as meat, fish, chicken, milk products, and beer are relatively poor iron sources.

Iron Transport

The plasma is the chief medium for the transport of iron, which is carried firmly bound to a plasma protein, probably globulin. It is, however, uncertain whether iron for haemoglobin synthesis is thus carried to the bone marrow as ferric ions, or as some pre-formed compound.

Iron is absorbed chiefly, and perhaps entirely, as ferrous ions, but the oxygen tension of the plasma is sufficient for the immediate conversion of the ions to the ferric state. The plasma iron is probably bound to the serum globulin as a ferric hydroxide complex.

Powell (1944) found the mean plasma iron of normal men to be 143 γ (S=24 γ), whereas the level in women was significantly lower, with a mean of 117 γ (S=27 γ). There was a significant reduction in the plasma iron of women during menstruation.

The plasma iron rises sharply during the absorption of a dose of iron from the intestine. The height of the increase depends upon the rate of deposition of iron in the tissues as well as upon the rate and amount of intestinal absorption, but plasma iron curves can be used to demonstrate whether iron is in fact being absorbed, and to obtain a rough idea of the relative absorption of different compounds. Moore and others (1939) have shown that the rise in the iron content of the plasma during absorption is not preceded by an increase in the iron content of the thoracic duct lymph, so that iron must pass directly from the intestinal mucosa into the blood stream.

The plasma iron is reduced below normal in patients with iron deficiency, and also during periods of active erythropoiesis such as occur after acute haemorrhage or during the cure of pernicious anaemia. Conversely, the plasma iron is usually high when erythropoiesis is depressed, as in untreated pernicious anaemia or hypoplastic anaemia. The level is very variable in haemolytic anaemias, depending upon the balance between red cell destruction and haemoglobin synthesis.

Easily split-off iron.—About 5 per cent of the blood iron can be easily dissolved by digestion with decinormal hydrochloric acid, and it was believed until recently that this "easily split-off iron" might be of importance in iron transport. Sixty to seventy per cent of the easily detached iron is derived from the red blood cells, but if the haemoglobin is converted into carboxyhaemoglobin this iron can no longer be removed by acid digestion. Legge and Lemberg (1941) have shown that this fraction is an artefact—when red blood cells are digested with acid, oxygen is liberated from its combination with haemoglobin and oxidizes a variable amount of the corpuscular haemoglobin into open-ring compounds similar to the iron-containing bile pigments, from which iron is easily removed by acid digestion. The remainder of the easily detached iron is derived from bile pigment-haemoglobin, blood catalase, and the plasma iron.

Iron Storage

It is evident from clinical observation that the body normally holds a considerable reserve of iron readily available for haemoglobin synthesis. A patient who, through acute haemorrhage, has lost a third or more of his circulating red cells may rapidly regain a normal haemoglobin level without any assistance from iron therapy, although the loss of 700 mg. or more of iron could scarcely be replaced from the diet within six months. We have little knowledge of the size of this available iron reserve. The greater part of the body iron is normally circulating as haemoglobin, and perhaps half of the balance is in the form of myohaemoglobin and cell-enzymes such as cytochrome, and is not available for haemoglobin synthesis. Table III shows Hahn's (1937) estimate of the iron

TABLE III

PERCENTAGE DISTRIBUTION OF THE TOTAL BODY IRON OF A NORMAL DOG (FROM THE DATA OF HAHN, 1937)

Circulating iron	Available reserves	Non-available iron
Blood haemoglobin.....57%	Liver, spleen, and marrow .....15% Elsewhere ...5%	Myohaemoglobin 7% Parenchyma iron.....16% (cell enzymes, etc.)
Total 57%	20%	23%

distribution in a normal dog. On a similar basis a normal man would have an available iron reserve of about 850 mg., sufficient to replace about one-third of his circulating haemoglobin.

The greater part of the iron reserve is in the liver, spleen, and bone marrow. Much of the iron in the marrow is present as haemoglobin in formed or immature red blood cells, whereas the reserve iron in other tissues is probably held in the reticulo-endothelial cells.

Many attempts have been made to gain further knowledge of the iron reserves by injecting colloidal iron intravenously into animals, but it is at least possible that the animal treats iron in this form as a foreign particle rather than as a normal dissolved metabolite.

The chemical form of the iron reserves is still not completely understood. Haemosiderin, which histologically is so prominent a product of red-cell destruction, is certainly not the major iron reservoir. It is probably an intermediate stage or side product in the conversion of haemoglobin into bile pigment, but little is known of the chemical reactions involved.

Ferritin.—Iron-storing tissues contain a protein, apoferritin, capable of combining with as much as 23 per cent of its weight of iron. The iron is firmly held to the surface of the apoferritin molecule as small micelles of ferric hydroxide–ferric phosphate to make a protein, ferritin, of variable iron content. Both ferritin and apoferritin can easily be crystallized as the cadmium salt, and minute quantities of either can be detected by a precipitin reaction with an anti-apoferritin serum.

Studies with radio-active iron have shown that at least a part of iron which is injected is quickly converted into ferritin, and that when "tagged" red cells are lysed the liberated iron can be detected in ferritin. It seems probable that ferritin functions as the most important, though not the only, store of iron readily available for haemoglobin synthesis. It may also be the intestinal iron receptor *(vide supra)*. The chemical properties and functions of ferritin have been well reviewed by Granick (1946).

Some of the earliest studies with radio-active iron showed that the iron liberated by destruction of effete red blood cells was immediately re-used for haemoglobin synthesis in preference to the reserve iron stores (Hahn and others, 1942; Cruz and others, 1942). Greenberg and Wintrobe (1946) have pointed out that when a small quantity (about 5 mg.) of radio-active iron is injected into a normal individual, about 15 per cent of the dose is built into haemoglobin each day. If the injected iron merged with the whole available iron reserve the utilization should be only 2 to 3 per cent daily, whereas if it were used in proportion to the daily liberation of iron from effete red cells the daily utilization should be about 83 per cent. It therefore appears that about one-quarter of the iron reserves form a "labile iron pool" used for normal haemoglobin synthesis. Dubach and others (1946) have also shown that injected iron, and presumably, therefore, iron from recently lysed red cells, is used for haemoglobin synthesis in preference to the iron reserves.

Iron Excretion

McCance and Widdowson (1937a, 1938) first demonstrated that iron excretion in man is too small to play any significant part in the control of iron metabolism. They pointed out that the faecal iron output varies with and nearly equals the food iron, and that when iron is injected practically none is excreted. In one dramatic experiment (McCance and Widdowson, 1937b) they treated a polycythaemic patient with acetyl-phenylhydrazine and reduced his haemoglobin level from 168 to 47 per cent Haldane. They calculated that over 6 g. of iron was liberated from the lysed red cells, yet less than 0.5 per cent of this amount was excreted. More recently (1943) the same authors transfused 28 pints of blood into a patient with haemolytic anaemia in a hundred days. All this blood was haemolysed, liberating about 80 mg. of iron a day, yet the total daily iron excretion (5.2 mg.) was slightly less than the dietary intake (5.6 mg. per day), so that none of the iron freed by haemolysis can have been excreted.

Hahn and others (1939b) followed the fate of radio-active iron injected as gluconate into dogs. The urinary excretion was negligible, apart from a "spill-over" immediately after the injection, and the faecal excretion was as small in plethoric as in anaemic dogs. Any considerable haemolysis of red cells increased the faecal iron excretion, and the authors received the impression that most of the iron thus excreted was derived from the bile.

Biliary iron excretion.—Hawkins and Hahn (1944) have recently studied the biliary excretion of iron by establishing biliary fistulae in dogs. After determining the basal daily iron and bile pigment excretion they produced severe haemolysis with acetyl-phenylhydrazine. The biliary iron and pigment excretion rose in parallel, sometimes as much as tenfold, so that it was reasonable to conclude that the biliary iron, like the pigments, was derived from the breakdown of haemoglobin. The iron was probably a part of the pigments, for

on injected intravenously was not excreted in the bile.

Hawkins and Hahn concluded from their experiments that about 3 per cent of the iron freed by haemolysis was excreted in the bile, and that the remainder was stored or re-used for haemoglobin synthesis.

Urinary iron excretion.—The importance of the urinary iron excretion is still uncertain. The careful analyses of Barer and Fowler (1937) suggest that the normal daily excretion of iron in the urine of men is 0.4 mg. and in women 0.5 mg.—a substantial proportion of the total daily loss. Other workers have regarded the urinary iron excretion as negligible, and derived from cell debris rather than from ionic transfer across the glomerular membrane.

Daily Iron Requirements

The minimum daily iron requirements are evidently equal to the total loss from the body by excretion in the bile and urine, desquamation of cells, and haemorrhage. The necessary dietary intake, in order to allow for unabsorbed iron, must of course exceed these physiological requirements, but a calculation of the latter provides a basis for comparing the needs of different classes of individuals.

Normal men.—An average man has 6 litres of blood with a haemoglobin content of 15.5 g. per 100 ml., so that his total circulating haemoglobin is 930 g. If we take the average life of the red cell to be 120 days, the daily breakdown of haemoglobin is 930/120=7.75 g., containing 26 mg. of iron (1 g. of haemoglobin contains 3.34 mg. of iron). If we assume that man, like the dogs of Hawkins and Hahn (1944), excretes 3 per cent of the liberated iron in the bile, the daily loss by this route is 0.8 mg. Urinary iron excretion, according to Barer and Fowler (1937), amounts to 0.4 mg. daily, giving a total iron excretion of 1.2 mg. per day (Table IV).

TABLE IV

DAILY IRON LOSSES OF NORMAL MEN AND WOMEN

	Men (mg.)	Women (mg.)
Biliary excretion	0.8	0.6
Urinary excretion ..	0.4	0.5
Menstruation	—	1.0
Total	1.2	2.1

Normal women.—A similar calculation can be made for normal women, but the iron loss of menstruation must be added. McCance and Widdowson (1937a) found by direct analysis that normal women lost 10–40 mg. of iron in each menstrual period, so that the average loss can be expressed as about 1 mg. per day over the whole menstrual cycle. A woman must therefore replace nearly twice as much iron each day as a man (Table IV), and even moderate menorrhagia may raise the requirements to 5 or 6 mg. daily.

Child-bearing.—Davidson and Fullerton (1938) calculated that a pregnant woman contributes 400 mg. of iron to the foetus and 150 mg. to the placenta and uterus. A blood loss in parturition of about 500 ml. accounts for a further 175 mg. of iron, making a total of 725 mg. The greater part of this iron drain occurs towards the end of pregnancy when the foetus is laying down most of its iron stores, but for convenience of comparison the iron loss may be expressed as an average of 2.7 mg. per day throughout the pregnancy. The total iron demands of a pregnant woman are thus about 3.8 mg. daily—nearly twice as much as a non-pregnant woman needs.

The iron drain of lactation is comparatively light, about 1.5 mg. a day, but when, as often happens, menstruation recommences during lactation the total iron loss is almost as great as in pregnancy.

Children and adolescents.—The increasing blood volume and tissue iron of children makes a considerable addition to their iron needs. Heath and Patek (1937) calculated the annual iron requirements for the increase in blood volume and parenchyma iron in each year of life; their figures make no allowance for any increase in the available iron stores. The additional iron thus laid down in the first year of life amounts to 200 mg., but the figure falls to 100 mg. during the third year and does not rise again appreciably until the ninth year, when the growth rate begins to increase. The amount of iron laid down annually then begins to rise to a maximum of 350 mg. during the seventeenth year, and then falls to zero as growth stops at about the twenty-first year. Thus the extra iron demands of growth never exceed 1 mg. a day, but even this is a substantial addition to the needs of a menstruating girl.

Clinically it appears that the iron needs of children are greater than these calculations admit, and it may be that their greater metabolic rate increases their iron needs.

Iron Losses from Haemorrhage

The reserve powers of the bone marrow are great enough to cope with haemorrhage at a rate of at least 150 ml. of blood a day, provided enough iron is available. A normal man replaces the red cells of 50 ml. or so of blood every day and only uses about one-quarter of his total marrow capacity, but his daily iron loss is only of the order of 1 ml. a day—the iron content of 2 ml. of blood. Even if the amount of iron absorbed from an ordinary diet were 20 mg. a day, a very improbable figure, the marrow would not receive enough iron to double its output. It is not, therefore, the loss of blood which prolongs the anaemia of haemorrhage, but the loss of iron.

Acute haemorrhage.—The blood loss of acute haemorrhage not exceeding one-third of the blood volume is usually quickly made good with the aid of the body's iron reserves, but it is well to remember how enormous is the iron loss in relation to the potential dietary intake. A pint of blood contains about 90 g. of haemoglobin and 300 mg. of iron, and even if the diet were capable of yielding an extra 5 mg. of iron a day in excess of a woman's normal requirements (2 or 3 mg. daily), it would take two months to replenish the iron reserves.

When repeated acute haemorrhages reduce the haemoglobin to below 50 per cent of its normal value the iron reserves will certainly be exhausted before recovery is complete, and unless extra iron is given the final rate of recovery will be limited by the available iron of the diet to something less than 2 per cent per week.

It is evident that iron therapy may be given with advantage during convalescence from acute haemorrhage, for with adequate dosage the body can absorb and utilize more than 50 mg. of iron a day. The idea is familiar to physicians, who most often deal with acute haemorrhage from peptic ulcer, often complicated by previous iron deficiency from chronic haemorrhage, but surgical and obstetrical patients also would probably benefit from iron therapy.

Chronic haemorrhage.—Relatively slight chronic haemorrhage greatly increases the iron losses of the body. Normal blood contains about 50 mg. of iron per 100 ml., so that the loss of only 10 ml. of blood a day increases the iron demands of a man fivefold. The drain of iron may to some extent be diminished by the development of anaemia, for the haemoglobin level and iron content of the blood fall in parallel.

It is uncertain how much haemoglobin iron is reabsorbed when chronic bleeding occurs into the small intestine. The question is of great importance in tropical countries, where iron deficiency from hookworm infestation is a major cause of ill-health. A load of 100 hookworms is only moderate, and will not always cause anaemia yet, if we accept the usual estimate, each worm withdraws 0.5 ml. of blood a day, making a total iron loss of 25 mg. daily. With really heavy infestations of 1,000 or more worms anaemia is almost inevitable, but even if the blood haemoglobin level is reduced to 3 g. per 100 ml. the blood withdrawn by the worms will contain 50 mg. of iron each day. It is scarcely possible for the whole of this deficiency to be replenished from the diet, so that unless the worms are less voracious than is commonly believed a great part of the haemoglobin iron must be reabsorbed from the intestine.

If, on the other hand, enough iron is supplied the reserve function of the bone marrow is sufficient to maintain the haemoglobin level in spite of chronic haemorrhage, unless prolonged anaemia has led to marrow hypoplasia.

Iron Therapy in Anaemia

It is possible to estimate the actual utilization of iron during recovery from anaemia if the changes in blood volume as well as in haemoglobin level are known. Some examples of such calculations are given below in order to emphasize how great is the amount of iron used in raising the haemoglobin to normal levels.

Iron-deficiency anaemias.—Table V shows the blood volume and haemoglobin level of a young woman suffering from a simple iron-deficiency anaemia. During the first nine days of treatment the total circulating haemoglobin increased by 90 g., corresponding to 300 mg. of iron, an increase of 50 per cent. The blood count did not change during the first four days of treatment, so from the fifth to the ninth day 60 mg. of iron were

TABLE V

TOTAL HAEMOGLOBIN VALUES IN A WOMAN WITH IRON-DEFICIENCY ANAEMIA

Day of treatment	0	9	23	49
Haemoglobin g. per 100 ml.	6.7	8.5	10.5	13.3
Plasma volume ml. ..	2,120	2,250	2,410	2,100
Blood volume ml. ..	2,830	3,310	3,700	3,690
Total haemoglobin g.	190	280	390	490

Patient's weight: 50 kilogrammes.

being utilized for haemoglobin synthesis each day. Since the anaemia was due to a complete exhaustion of the patient's iron reserves, all of this iron must have been absorbed from the intestine. Exsiccated ferrous sulphate was being given in a dose of 1.8 g. (27 gr.) daily, containing 600 mg. of iron, 10 per cent of which was being absorbed and utilized. At the end of seven weeks the haemoglobin had still not quite reached a normal level, but the total amount in circulation had increased by 300 g., corresponding to 1,000 mg. of iron. Over the whole period of treatment the patient had utilized iron at an average rate of 20 mg. a day.

Before this patient was treated she had a normal plasma volume and, consequently, a low blood volume, so that her haemoglobin level underestimated her total iron deficiency. Her cure demanded not only a return of the haemoglobin level to normal, but also an increase of the total red cell volume to give a normal blood volume. Thus whereas during the course of treatment her haemoglobin level rose to 100 per cent above its original level, the total circulating haemoglobin increased by 160 per cent. These figures illustrate how great may be the discrepancy between the true iron utilization and the apparent utilization calculated simply from the haemoglobin level without regard to the changing blood volume.

Pernicious anaemia.—Theoretically, iron should not be necessary in the treatment of pernicious anaemia, for the haemoglobin level was once normal, and nearly all the iron from the missing red cells is retained in the body. In practice, however, iron deficiency, as shown by a mean corpuscular haemoglobin concentration below 32 per cent, is often present when the anaemia is first diagnosed, or else develops during treatment. Iron deficiency is so common in women that in them it must often precede pernicious anaemia, but the frequent development of iron deficiency during the treatment of pernicious anaemia in men is not so readily explained. Dubach and others (1946) have shown that recently injected radio-active iron is more readily utilized than the tissue iron reserves during the cure of pernicious anaemia, and it may be that much of the great excess of iron in the tissues is not readily available for haemoglobin synthesis.

Table VI indicates the great amount of iron which may be utilized during the cure of a case of pernicious anaemia. The patient, an old woman, had a total blood volume which was low for her weight, though the plasma volume was definitely above the normal. Her total circulating haemoglobin rose during treatment from 130 to 530 g., an increase utilizing 1.34 g. of iron. The patient was a very small woman, weighing only 45 kilos, and on the same basis a man of 70 kilos with pernicious anaemia of the same severity would utilize about 2 g. of iron in raising his haemoglobin to normal.

TABLE VI

TOTAL HAEMOGLOBIN VALUES IN A WOMAN WITH PERNICIOUS ANAEMIA

Day of treatment	0	50
Haemoglobin g. per 100 ml. ..	3.9	14.0
Plasma volume ml.	3,070	2,300
Blood volume ml.	3,470	3,800
Total haemoglobin g.	130	530

Patient's weight: 45 kilogrammes.

These figures emphasize the necessity for watching for the development of iron deficiency during the treatment of pernicious anaemia, or, if frequent haematocrit investigations are not possible, of supplementing the liver therapy with adequate doses of iron.

The Anaemia of Infection

Infections in man are commonly complicated by a normocytic or slightly microcytic anaemia which is very resistant to treatment until the infection is cured. The frequency of hypochromia in this type of anaemia suggests that it is at least in part due to some disturbance of iron metabolism, and Cartwright and others (1946a, b) have recently done much to confirm this view. They found that the plasma iron was markedly diminished during infections, and that the hypoferraemia persisted until the infection was cured, even though there was no anaemia. The fall in the plasma iron might be evident within two or three days of the onset of an acute infection. When 1 g. of ferrous sulphate was given by mouth the plasma iron level did not rise, although in normal subjects such a dose would increase the level twofold. Even an intravenous injection of iron disappeared from the blood with abnormal rapidity, so it seemed that the anaemia was due at least in part to a deviation of iron from the bone marrow to some other site—probably the infected tissues.

Hypoferraemia followed by anaemia was found when staphylococcal or sterile turpentine abscesses were produced in dogs, but neither staphylococcal toxin nor killed typhoid vaccine produced these changes.

Hahn and others (1946) have also shown that anaemic dogs utilize radio-active iron much more

slowly after the induction of a sterile turpentine abscess than before.

We still do not know whether this deviation of iron from blood formation to infected tissues is a feature of the defence mechanism or simply a toxic reaction.

Summary

There is no evidence of excretion of iron by the body except in very small amounts, and the iron balance must therefore be maintained by a regulation of iron absorption. The greater part of the iron freed from broken-down red cells is re-used for haemoglobin synthesis, and only about 1 mg. a day is excreted in the bile and urine. Even the iron losses of normal menstruation, pregnancy, and lactation do not raise the average daily loss above 4 mg.

Iron absorption from the intestine varies, in accordance with the body's needs, from the 1 mg. or so a day necessary to maintain the iron balance of a normal man, to the 50 mg. or more a day which may be absorbed and utilized during intensive treatment of iron-deficiency anaemia. The amount of iron absorbed is determined by the iron level in the cells of the intestinal mucosa, where a protein receptor, probably ferritin, can receive iron from the intestinal contents and yield it to the plasma. Iron is transported by the plasma to the tissue stores, and the equilibrium between the iron level in the stores, the plasma, and the intestinal receptor determines the saturation of the last and the degree of iron absorption from the food. Anaemia *per se* has no influence on iron absorption.

Iron can be absorbed from the stomach and small intestine so long as the reaction is acid. Only ions are absorbed, so that the non-ionizable porphyrin iron of the food must be broken down by digestion or bacterial decomposition before it can be assimilated. It is possible that only ferrous ions can be absorbed, and they are certainly much more readily absorbed than ferric ions, but the latter are also more apt to form insoluble compounds in the intestine. The value of a diet as an iron source is largely determined by the amount of substances, such as phosphates, certain protein derivatives, and phytic acid, which form undissociated compounds with iron. As a general rule, however, an ordinary mixed diet suffices for a man if it contains a total of 5 mg. of iron, or for a woman or growing child if it contains 15 mg.

Some 60 per cent of the total body iron is circulating as haemoglobin in the blood, and a further 20 per cent is held in the tissues in a form potentially available for haemoglobin synthesis. The greater part of this storage iron is present as ferritin. Iron recently absorbed or liberated from destroyed red cells is used for erythropoiesis in preference to the iron reserves, and about one-quarter of the latter seems to be more immediately available than the rest.

Of the remaining body iron, about one-third is in myohaemoglobin and about two-thirds in such cell enzymes as cytochrome. None of this iron is available for haemoglobin synthesis even in extreme iron depletion, but beyond this scarcely anything is known of its metabolism.

The iron losses of even moderate haemorrhage are very heavy in relation to the body's usual needs. A man normally absorbs only a milligramme or so of new iron a day, whereas 100 ml. of his blood contains about 50 mg. of iron. A blood loss up to about one-third of the total blood volume can be replaced from the iron reserves if these are saturated, but any greater loss must be replaced from the diet, which if unsupplemented will allow a haemoglobin increase of only some 2 per cent per week. The iron loss of chronic haemorrhage can easily exceed the capacity of an ordinary diet, but if the bleeding is into the stomach or duodenum a part of the haemoglobin iron can be re-absorbed.

Recovery from severe anaemia may utilize 2 g. of iron, a variable amount of which can be taken from the body's reserves. At the one extreme, in iron-deficiency anaemia all the iron must be absorbed; at the other, in pernicious anaemia the iron reserves are theoretically adequate to raise the haemoglobin to normal, although in practice the whole reserves are apparently not always readily available.

The anaemia of infection is associated with a low plasma iron, and there is evidence that iron is deviated from blood formation to the site of infection.

My thanks are due to Sir Lionel Whitby, Regius Professor of Physic in the University of Cambridge, for his advice and assistance in the preparation of this review.

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THE ERROR OF COLOUR MATCHING WITH HALDANE'S HAEMOGLOBINOMETER

BY

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(RECEIVED FOR PUBLICATION, SEPTEMBER, 1947)

Haldane and Smith (1899–1900), in the description of their well-known technique for the determination of haemoglobin in blood, realized that the comparison of the tints of solutions of carboxyhaemoglobin contained in tubes may be inaccurate. They wrote as follows (p. 334): ". . . when the tints are really equal the tubes may look unequal, the apparent inequality being, however, in the other direction when they are transposed. This commonly affects not merely the depth, but also the quality of the tints. Errors of such a kind are avoided by repeatedly transposing the tubes during the comparison." In the same communication (p. 333), they stated: "The points at which there was just too little, and just too much, water were noted, and the mean taken as the correct result."

This advice, to judge from a recent publication, is often overlooked. Macfarlane (1945, p. 65) inquired of sixty "competent observers" engaged in haemoglobin surveys for the Medical Research Council as to the precise manner in which they used the instrument. Thirty-nine stated that they transposed the standard and unknown tubes as advised by Haldane, whereas seventeen did not transpose the tubes, and four did not reply to the inquiry. It appeared further that, of these sixty observers, only five adopted Haldane's advice in its entirety.

We have investigated the accuracy with which two observers (J.L.D. and E.C.T.) could match the tints of solutions of carboxyhaemoglobin under different experimental conditions. The results of these experiments are in the course of publication elsewhere. They may be stated briefly as follows:

(*a*) When two identical, ungraduated, haemoglobinometer tubes containing solutions of carboxyhaemoglobin are viewed side by side, and touching against a background of an illuminated ground-glass screen, the observer may judge the solution on the right to be "too dark" (that is, strong) when, in fact, it is the weaker of the two.

(*b*) The error alters as the distance between the observer's eyes and the comparison tubes alters.

(*c*) At any one distance the error changes if the intensity of illumination is changed, or if a daylight bulb is substituted for a "clear" bulb as the source of light.

In the present series of experiments, the errors of twenty observers (six pathologists, four senior technicians, and ten medical students) were investigated.

Plan of Research

The following groups of experiments were carried out.

(*a*) Each observer made a series of comparisons in daylight.

(*b*) Each of the ten students made two series of comparisons, (i) with the eyes at 40 cm., and (ii) with the eyes at 125 cm. from the comparison tubes, the source of light being a "clear" electric bulb set up as described below.

(*c*) Conditions were found in which the student observers could compare the tints of solutions of carboxyhaemoglobin with considerable accuracy.

The Experimental Method

A series of nine dilutions of carboxyhaemoglobin was made up from a 2 per cent stock solution of blood whose haemoglobin content was 100 per cent (Haldane). The tint of the solution labelled "1" approximately matched a Haldane standard. The remaining eight solutions differed from one another and from the solution "1" by steps of 2 per cent. The strongest solution was labelled 1.08, and the weakest 0.92.

The same two ungraduated haemoglobinometer tubes of average internal diameter 6.60 and 6.63 mm. were used in all these experiments, and in every comparison of tints the same tube always occupied the left-hand position. The tubes were not transposed. The tubes were filled by an experimenter with any pair of the nine available solutions of carboxyhaemoglobin. The observer's part in the experiment was solely to report whether, in his opinion, the solution in the right-hand

tube was "too strong" or "too weak" relative to that in the left-hand tube. If he could not distinguish between them, he reported a "match." The tubes were set up side by side and touching in a cork holder. The solutions were presented for comparison in a random order, and the number of comparisons which made up an experiment was left to the discretion of the experimenter, who was guided by the observer's replies and continued the experiment until he was sure that a given difference in concentration between the solutions in the two tubes could be recognized correctly, and with certainty, by the observer.

From the observations, the ratio of the concentrations of the solutions in the right- and left-hand tubes which gave the observer an equal chance of recognizing a difference in tint correctly was calculated. This was called the "50 per cent ratio." Thus, if the 50 per cent ratio is 1, it means that the observer did not bias his observations in favour of either tube. On the other hand if it is 0.95, it means that the observer judged the solution in the right-hand tube to be "strong" when, in fact, it was "weak." The value of the 50 per cent ratio, therefore, is a measure of an observer's error in tint discrimination.

The method of calculation is based on that used for determining the LD50 for a drug (*cf.* Gaddum, 1933), Behrens's (1929) calculation to allow for the smallness of the sample being employed. When the normal equivalent deviation (N.E.D.) of the percentage of correct observations at each ratio tested is plotted against the ratio concerned, the points lie on or near a straight line. The ratio corresponding to zero N.E.D. represents the ratio at which the observer has an equal chance of discriminating between the tints of the two solutions. The standard deviation (S.D.) of the 50 per cent ratio was calculated from formula 13 on p. 27 of Gaddum's (1933) paper. The application of the method is described in detail in another communication (D'Silva and Turton).

Comparisons in Daylight

Each of the twenty observers undertook a series of comparisons as described above, in a laboratory lighted by daylight. The observer stood and held the tubes in the cork holder against a ground-glass screen placed in front of the window at eye level. The intensity of illumination varied, but in no case would it have been deemed too low for haemoglobinometry in a pathological laboratory.

Having permitted the observer to choose the distance between the tubes and his eyes, this distance was determined by the experimenter as follows: two cardboard scales marked by vertical lines at intervals of 5 cm., the one being slightly wider than the other, were arranged parallel with the observer's line of sight. One was about 30 cm. and the other 40 cm. from that line. The "zero distance" on each scale was some 2 cm. nearer to the observer than the ground-glass screen, and coincided with the usual position of the comparison tubes when they were held near the screen. Unknown to the observer, the experimenter observed the distance at which the tubes were held from the eyes for each comparison. The arrangement of the graduated scales eliminated any error due to parallax.

Two sets of results were obtained from this series of experiments: (*a*) the error of comparison of each observer; and (*b*) the least, the mean, and the greatest distance between the tubes and the observer's eyes for each set of comparisons.

The error of each observer is recorded in Table I. It will be seen that in each group some observers are fairly accurate and some are inaccurate. The errors of both groups are similar in spite of the fact that one group of observers was trained to compare these tints and the other was untrained. Some of the student observers had used the Haldane method as a class experiment, so that to achieve the degree of accuracy shown by them required little or no training.

TABLE I

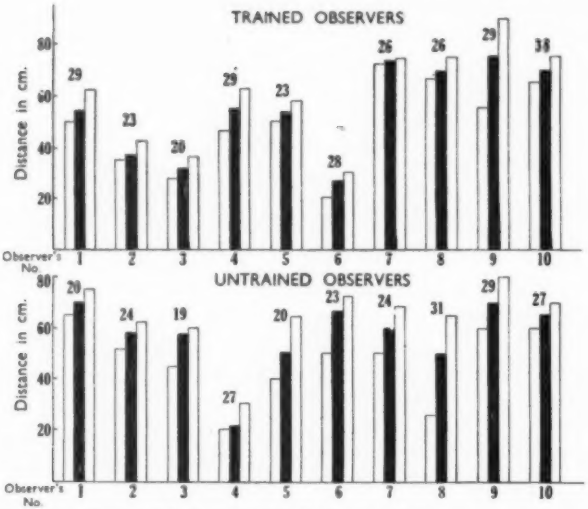
ERRORS MADE BY TWENTY OBSERVERS IN COMPARING THE TINTS OF SOLUTIONS OF CARBOXYHAEMOGLOBIN IN DAYLIGHT

Trained observers		Student observers	
Initials and no.	50% ratio and 2S.D.	Initials and no.	50% ratio and 2S.D.
1. A.E.K.-S.	0.997 ± 0.017	1. R.Z.	0.970 ± 0.009
2. B.C.C.	0.969 ± 0.008	2. P.J.L.	0.969 ± 0.012
3. M.B.McI.	0.969 ± 0.008	3. G.C.H.C.	0.968 ± 0.014
4. A.McG.	1.033 ± 0.010	4. C.C.M.	0.964 ± 0.014
5. R.J.S.	0.960 ± 0.008	5. P.H.S.	0.958 ± 0.013
6. d'A.K.	0.957 ± 0.012	6. A.J.W.	0.957 ± 0.015
7. G.D.	0.956 ± 0.014	7. R.H.G.B.	0.945 ± 0.012
8. H.I.C.	0.955 ± 0.011	8. D.M.	0.937 ± 0.016
9. D.A.L.	0.941 ± 0.016	9. D.E.W.	0.936 ± 0.026
10. G.E.M.	0.873 ± 0.018	10. W.R.C.B.	0.921 ± 0.009
Average 0.961		Average 0.953	

The diagram shows the mean distance (black area), the least distance, and the greatest distance at which comparisons of tint were made. In the student group, 50 cm. or above was the usual distance, as it was in the group of trained observers, except for one observer in the former group and three in the latter who made their comparisons at distances nearer than 50 cm. It was not possible to correlate the observer's error with the distance at which he made the comparisons of tint. The distance of comparison usually varied within fairly narrow limits except in the case of D.E.W. (student group) and D.A.L. (trained group), whose comparison distances were very variable.

Comparisons in Artificial Light

The source of light was a 60-watt "clear" bulb mounted inside a black box, the front of which was covered except for a circular aperture 4 in. in diameter placed opposite the bulb. A ground-glass screen was set up



The mean distance (black rectangle), the least, and the greatest distance at which observers made their comparisons in daylight. The observers' numbers correspond to those in Table I. The number of observations is given at the head of each group of three rectangles.

3.5 ft. from the bulb (illumination of 10 f.c. approximately) and the comparisons of tint were made against it. The room was darkened apart from the light referred to above. The comparisons were made as described previously.

Each of the student observers carried out a set of comparisons with the eyes at (*a*) 40 cm. and (*b*) 125 cm. from the tubes. The results are in Table II.

In every subject, except P.J.L., R.Z., and W.R.C.B., the 50 per cent ratio changed when the distance between the eyes and the comparison tubes was altered from 40 to 125 cm. The change in distance can affect the error of comparison in three ways: (*a*) the 50 per cent ratio may be unchanged; (*b*) it may become less; or (*c*) it may become greater. Of the observations made at 40 cm., only three observers (R.H.G.B., C.C.M., and P.H.S.) had 50 per cent ratios not significantly different from 1. All the observers (except R.Z. at 40 cm.; *cf.* Table II) were consistent in their observations, though only three were accurate. Their results showed that the precise difference in concentration between the solutions in the two tubes which led to difficulty in distinguishing between the tints was different for different observers.

Conditions under which Accurate Comparisons were made by some Observers

In this series of experiments (results in Table III) we tried to find conditions under which the observers' 50 per cent ratios would not differ significantly from 1. Three of them (R.H.G.B., C.C.M., and P.H.S.) made accurate tint comparisons at 10 f.c with the eyes 40 cm. from the tubes (*cf.* Table II). The results of the other seven observers show that when the distance of comparison was altered from 40 to 125 cm., in three (D.M., A.J.W., and G.C.H.C.) the 50 per cent ratio increased, in one (D.E.W.) it diminished, and in three (P.J.L., R.Z., and W.R.C.B.) it was unchanged.

Of the three observers for whom the 50 per cent ratio increased, in two it changed from less than 1 to more than 1. For these two (D.M. and A.J.W.) we found empirically that accurate tint discrimination

TABLE II

ERRORS OF COMPARISON OF TEN STUDENT OBSERVERS WITH AN ILLUMINATION OF ABOUT 10 F.C. PROVIDED BY A 60-WATT "CLEAR" BULB

Observer	50% ratio and 2 S.D.	
	40 cm.	125 c.m.
R.H.G.B.	0.999 ± 0.012	1.044 ± 0.016
C.C.M.	1.005 ± 0.012	1.056 ± 0.017
P.H.S.	1.006 ± 0.015	0.950 ± 0.010
P.J.L.	0.980 ± 0.011	0.977 ± 0.013
D.M.	0.968 ± 0.009	1.027 ± 0.022
A.J.W.	0.967 ± 0.015	1.057 ± 0.014
D.E.W.	0.955 ± 0.012	0.912 ± 0.015
R.Z.	0.954 ± 0.040	0.940 ± 0.017
W.R.C.B.	0.945 ± 0.014	0.958 ± 0.013
G.C.H.C.	0.924 ± 0.022	0.960 ± 0.014

TABLE III

CONDITIONS OF ILLUMINATION AND DISTANCE WHICH ALLOWED SEVEN UNTRAINED OBSERVERS TO COMPARE THE TINTS ACCURATELY: C = "CLEAR" BULB; D = "DAYLIGHT" BULB

Observer	Illumination in f.c.	Dist. in cm. of eyes from tubes	50% ratio and 2S.D.
R.H.G.B.	C 10	40	0.999 ± 0.012
C.C.M.	C 10	40	1.005 ± 0.012
P.H.S.	C 10	40	1.006 ± 0.015
D.M.	C 10	50	0.991 ± 0.009
A.J.W.	C 10	60	0.995 ± 0.014
D.E.W.	C 100	40	0.991 ± 0.009
G.C.H.C.	D 10	85	1.007 ± 0.014

was achieved when the distance of comparison was 30 cm. and 60 cm., respectively. On the same basis, G.C.H.C. presumably needs a longer comparison distance than 125 cm. (*cf.* Table II), but comparisons made at distances greater than 125 cm. are less accurate. When illumination was provided by a daylight lamp, however, G.C.H.C. was accurate at a comparison distance of 85 cm. (Table III).

In the case of D.E.W., the 50 per cent ratio diminished as the comparison distance increased and was always less than 1 at 10 f.c. We found no distance at which this observer could make accurate comparisons at 10 f.c. At 100 f.c., however, he made accurate comparisons when his eyes were 30 cm. from the tubes.

The remaining three observers (P.J.L., R.Z., and W.R.C.B.), for whom a change in comparison distance was without significant effect on the 50 per cent ratio, were not dealt with further. P.J.L. had only a small error (2 per cent ± 1 per cent) in his 50 per cent ratio at 40 cm. and it was felt that any significant change in this in the direction of greater accuracy would be difficult to establish. W.R.C.B. had a large error, but we could not find any conditions under which his 50 per cent ratio was near 1. It was later discovered that he had uncorrected astigmatism which may have contributed towards his inaccuracy. R.Z. at 40 cm. (Table II) had a wide scatter in his observations (as is shown by a high S.D.), and though he was more consistent at 125 cm. he was just as inaccurate.

Commentary

Three points arise from this work in connexion with the determination of haemoglobin.

(*a*) All but one of our trained observers made appreciable errors in comparing the tints of solutions of carboxyhaemoglobin. They were no more accurate than our untrained observers and, as we have shown previously, the inaccuracy is not due to instrumental errors. It seems probable that it is the result of the make-up of the individual's visual apparatus. None of our observers was aware, during the course of the experiment, of any inaccuracy of comparison, so for accurate work it would be advisable for workers who do not transpose the haemoglobinometer tubes during their tint-matching procedure to undertake experiments to determine the magnitude of their errors.

(*b*) Macfarlane's recent communication (1945, p. 67) shows that six different methods of haemoglobinometry were in use by 56 observers, namely: (i) standard always kept on the left, (ii) standard always kept on the right, and (iii) standard first on one side and then on the other. All the observers using these three methods took the first observation of "match" to be the true match point. The observers (iv), (v), and (vi) used the methods (i), (ii), and (iii), respectively, in their tint-matching procedure, but took the mean of the last observation of "strong" and of the first observation of "weak" as the true match point.

We can illustrate the possible errors which can be made by an observer by considering one with a 50 per cent ratio of 0.90, that is, one who judges the right-hand tube to be 10 per cent stronger than it is. Let us assume that he uses the experimental procedures (i) to (vi) (above) in turn, and further that the true match should be at 100 on the Haldane scale.

If he keeps the standard always on the left, he will match at 110. If he keeps the standard always on the right, he will match at 90 for the same reason. If he uses (iv) or (v) his match points are more likely to be 112 and 92, respectively, because the difference between his last observation of "strong" and his first observation of "weak" will be 4 per cent or 6 per cent on the Haldane scale. In each of these four experiments he will have a large error solely because of his inaccuracy in tint matching.

Now consider the situation when the same observer examines the unknown solution first on the right of and then on the left of the standard. Assume that in the course of the experiment, dilution has reached the "90-mark." When the standard is to the left of the unknown, the latter will be recorded as strong. When the positions of the tubes are reversed, however, a match may be recorded because of the observer's 10 per cent error. The observer will be faced with a dilemma in which the same solution appears to be strong when it is viewed on one side of the standard, and a match when it is viewed on the other side. Two courses only are open to him. Either (*a*) he can record his judgment as a non-committal "match" and continue his observations, finally taking the mean of the readings at which he judged the unknown to be just too strong and just too weak as the true match point (*cf.* procedure (vi), above); or (*b*) he can judge the dilution at which the unknown appears as strong, when it is viewed on one side of the standard, as it appears weak when it is viewed on the other, and call this the true match point. The latter procedure introduces uninvestigated factors concerning judgment and memory for differences of tint and so, at present, must be regarded as unreliable. It is likely, therefore, that the observer will obtain the most accurate results when he examines the unknown first on the right of and then on the left of the standard and takes the mean of the last

observation when the unknown appeared strong whether it was viewed on the right or the left of the standard, and the first observation when the unknown similarly appeared weak as the correct match point. This is the procedure which was advised by Haldane.

(*c*) To obviate the above inaccuracies, an observer should discover standard conditions of illumination and distance which enable him to compare the tints accurately, and use these conditions whenever he is determining the amount of haemoglobin in blood.

Summary

1. There was no difference in the accuracy with which ten trained and ten untrained observers matched the tints of solutions of carboxyhaemoglobin in daylight. The distance at which they made their comparisons was usually over 50 cm. and varied only within narrow limits in eighteen out of twenty observers.

2. The accuracy of seven out of ten untrained observers changed as the distance between their eyes and the comparison tubes changed.

3. Conditions of illumination and distance of comparison were found for seven out of ten untrained observers which enabled them to make accurate comparisons of tint.

4. The application of these observations to haemoglobinometry is discussed.

We are indebted to Professor Hartridge for his advice in the preparation of this paper, and to our observers for their co-operation.

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J. clin. Path. (1948), 1, 73.

RAPID AND ECONOMICAL RHESUS TESTING: A TRIAL OF CHOWN'S CAPILLARY TUBE METHOD

BY

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(RECEIVED FOR PUBLICATION, DECEMBER 30, 1947)

Introduction

It is now firmly established that immunization by the Rhesus factor plays a predominant part in the pathogenesis of haemolytic disease of the newborn Levine and others, 1941a and b), and that treatment by early induction of labour and transfusion or exsanguination transfusion will reduce the mortality of affected infants to about one quarter of that found in an untreated series (Diamond, 1947). It has also been shown that a single transfusion of Rhesus-positive blood will sensitize about half of all Rhesus-negative recipients (Moloney, 1945 ; Diamond, 1947 ; Hattersley, 1947), and that Rhesus-positive babies born to previously sensitized Rhesus-negative mothers are usually affected by a severe form of haemolytic disease. It is therefore essential to determine the Rhesus group of all female recipients of transfusions who are potential mothers, and to give only Rhesus-negative blood to Rhesus-negative recipients.

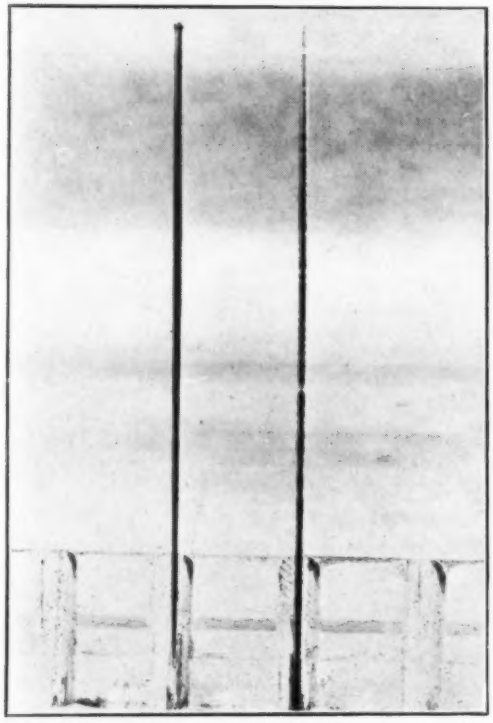
It is possible that the slide test, using the incomplete or blocking antibody (Diamond and Abelson, 1945), would be the method of choice, but unfortunately high-titre high-avidity sera of this type are not generally available in this country. Satisfactory sera derived from animals are troublesome to prepare. Sera of human origin containing the ordinary agglutinating antibody have thus to be used, but unfortunately sera with a high titre (1/32 or over), are seldom encountered, though there is less difficulty in obtaining sera with a titre of 1/8 or 1/16.

The standard technique, using precipitin tubes, is reliable but laborious ; it also requires scrupulously clean glassware and relatively large volumes of sera. This report is concerned with a recent trial of the method of Chown (Chown and Lewis, 1944, 1946), to which our attention was directed by Dr. Race.

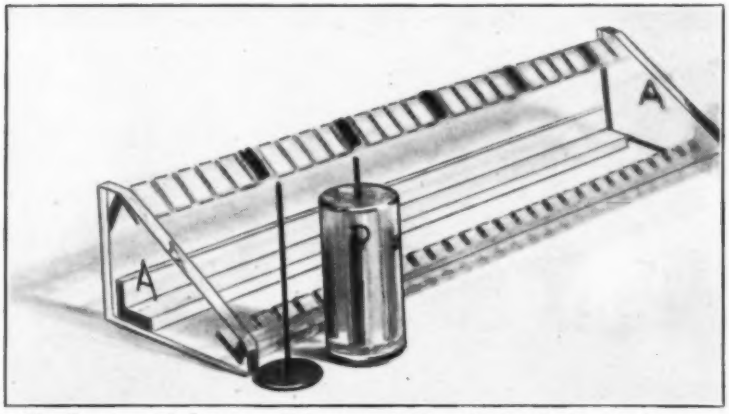
Method

In Chown's technique the cells to be tested are allowed to fall through a column of agglutinating serum contained in an inclined capillary tube of 0.3 to 1.0 mm. bore. If the cells are agglutinated they seem to become sticky and to adhere to the wall of the capillary in discrete clumps which can easily be seen by the naked eye, usually within ten minutes of starting incubation. Capillary tubes of about 0.5 mm. (0.3 mm. to 1.0 mm.) bore and about 7 cm. in length are required. These are easily drawn from 7 to 8 mm. diameter soft glass tubing and broken to approximate length between the fingers ; several hundred may be made in half an hour. A block of plasticine is also needed, but if a large series is to be done it is convenient to replace the plasticine by a container of soft paraffin and to employ a perspex rack or the heated rack described by Chown and Lewis (1946) to hold the capillaries. An anti-D agglutinating serum from which the anti-A and anti-B agglutinins have been absorbed is employed.

A capillary is dipped vertically into the serum until a column 10 to 20 mm. long has entered by capillarity. It is then removed and dipped into the unknown test cell suspension until a column of about the same length has entered (care must be taken to avoid air-locks), inverted, and the lower end (which did not enter the fluids) stuck into the plasticine at an angle of about 45° : or, if a rack is used, the lower end is plugged with vaseline and the capillary placed in the rack. The capillary is then incubated at 38° C. and inspected at intervals. In about half of all Rhesus-positive samples, segmentation of the cell column will be visible in 5 to 10 minutes ; this segmentation constitutes a positive reaction (Plate I*a*). Most positives are definite in 15 minutes, but a few develop only after about



(*a*).—The capillary test. Left-hand tube—negative reaction. Right-hand tube—positive reaction.



(*b*).—Perspex rack for tests. Polystyrene holder with handle for simultaneous washing of five samples of cells.

PLATE I.

30 minutes. A weak serum, or too dilute a cell suspension, delays the appearance of the positive reaction. If the cells are Rhesus-negative, there is no suggestion of segmentation, the cells forming a uniform smooth layer on the lower wall of the capillary. It will be noted that in cases of urgency, where the result has to be read within a short time, an occasional Rhesus-positive blood may be misread as negative (which is of minor importance), but never Rhesus-negative blood as positive.

The strength of the cell suspension is most important. A mixture of fresh oxalated blood with once to twice its volume of isotonic citrate solution is recommended by Chown; this gives a cell suspension of about 15 per cent and is very satisfactory. However, most antenatal patients have blood taken for a Wassermann reaction. It was decided to use the residue of these samples, after the serum had been removed, by shaking the clot and the extruded cells with 1 to 2 volumes of saline. Such suspensions were found to be satisfactory, though suspensions made by removing the extruded cells by decantation, and shaking with fresh saline, were not, for they were always too dilute and frequently contained fragments of clot.

A comparison between this capillary method and the standard tube method has been made, a total of 503 specimens being examined. Of these, three were rejected owing to gross haemolysis or infection, though two proved satisfactory in the tube technique; eight gave doubtful readings at the first trial of the capillary method, but became definite if a higher concentration of cells was used for the test; all these eight were over six days old. Altogether, there were 399 samples positive by both methods, of which 391 were clear-cut and obvious in the capillaries, and 101 samples negative.

On the basis of this comparison, it was decided that the capillary method was suitable for routine use, subject to certain limitations; first, the blood must be uninfected and not more than four days old; secondly, dilute suspensions of cells must be concentrated by centrifuging and resuspension; thirdly, doubtful reactions must be checked by the tube technique. The method has now been used for some months using anti-A, anti-B, anti-D, anti-C, anti-E, anti-c and anti-(C+D+E) sera, and so far the results have been unexceptionable if fresh suspensions of cells at 10 to 20 per cent concentration have been used. It has also been used to demonstrate both complete and incomplete antibodies in unknown sera, but so far insufficient experience has been gained to warrant its general adoption for the detection of antibodies, though it appears to be at least as sensitive as the tube technique. Chown (1947) informs us that in his hands it is more sensitive, and we have succeeded in obtaining clear-cut results with one batch of serum so weak that it was useless for the tube technique.

A statistical analysis of the frequency of the D-antigen in (1) the 500 samples described, (2) a series of blood donors, (3) the first 400 antenatal patients studied as a routine, and (4) a series of 2,000 cases studied by Race (1947) has been made, the four series, and some of their combinations, being analysed for homogeneity by the χ^2 method (Tables I and II).

TABLE I

Series	Rhesus-positive	Rhesus-negative	Total
1	399	101	500
2	124	35	159
3	341	59	400
4	1,658	342	2,000
Total	2,522	537	3,059

TABLE II

Comparison	χ^2	P
1 with 2 with 3	6.27	0.04
1 with 2 with 3 with 4 ..	7.15	0.07
1 with 3	4.5	0.038
(1 + 2 + 3) with 4	0.848	0.62
1 with 4	2.6	0.1
3 with 4	2.75	0.1

It is obvious that there is a difference of doubtful significance between the series 1 and 3. Since all Rhesus-negative subjects are tested twice, once with anti-D and once with anti-(C+D+E) serum, while many mothers of erythroblastotic infants have been transfused with blood from our own donors, it seems likely that the difference is due to chance.

Apparatus

If any number of tests are to be done, it is an advantage to have specially designed apparatus. We have used perspex racks similar to those advised by Berlin (1947). In our comparison of the two techniques we found that to avoid labelling tubes in

which cells were washed, blocks of translucent polystyrene plastic could be used; these were cylindrical, about 1¼ in. diameter and 2¼ in. long, with an axial hole to take a 20 S.W.G. brass wire. In each block five holes to carry precipitin tubes were drilled, each 2 in. long; the block was labelled with a letter, and each hole for a tube with a number. Labelling is best done with an ink containing Sudan Black B dissolved in a mixture of dichlorethylene and ethylene dichloride containing about 10 per cent Diakon (perspex) cement. The block fits a 50-ml. centrifuge bucket, and can be lifted out by the wire handle. The wall of the block is so thin that the column of red cells in the tube is easily seen, and after centrifuging the supernatant fluid can be sucked off and the cells resuspended without removing the tube from its holder (Plate I*b*). An apparatus for removal of supernatant fluids was also devised; a suction pump is attached to a small Büchner flask, from which passes a rubber tube attached to a Pasteur pipette; the lumen of the rubber tube is closed by a glass bead, and by pinching the rubber tube to one side of the bead a channel is formed.

Conclusion

The capillary method has been so satisfactory in our hands that we no longer use the tube method in routine Rhesus typing, but keep it for testing for agglutinins in unknown sera and for titrating sera. The capillary method requires little skill, the result usually being definite in fifteen minutes.

We are indebted to Dr. R. R. Race for drawing our attention to the method, to Dr. A. E. Mourant for supplying us with many of the antisera we have used, to the originator of the method, Dr. Bruce Chown, for his valuable advice, and to Mr. Tilman, assistant engineer to the hospital, for making apparatus to our design.

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APPENDIX

NOTE ON THE WORKING OF POLYSTYRENE ROD

BY

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The polystyrene plastic is brittle, and melts if machined carelessly or if it is not continuously cooled. Soap suds must be run over it continuously when machining or drilling. For turning, the rod is wrapped with a single layer of thick paper and held in a chuck and turned at 300 revolutions per minute. The tool must be of a good grade of tool steel, ground as for a side tool with nose slightly rounded off with a slight lip, and stoned to a fine edge; ordinary grinding does not produce a smooth finish. Light cuts must be taken and coolant used continuously.

For drilling, a drilling machine or a lathe with instantaneous feed on a loose headstock must be used, since swarf chokes the flutes of the drill and leads to local melting. The drill is ground as for brass and honed to a keen edge; it is fed lightly and cleared every 1/4 in. to 3/8 in., using a copious coolant.

As much material as possible should be left, especially where holes are close together.

J. clin. Path. (1948), **1**, 77.

A SIMPLIFIED PRICE-JONES TECHNIQUE*

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(RECEIVED FOR PUBLICATION, MAY, 1946)

The measurement of the mean diameter of the red blood cells is still one of the most important observations that can be made. Unfortunately the most accurate method of measurement by the technique introduced by Price-Jones is tedious and time-consuming. To trace the outlines of 500 cells, and then to measure the largest and smallest diameter of each, takes at least four hours. It also requires skill and practice. By the following technique, derived from that of Hynes and Martin (1936), and of Price-Jones as described fully by Mogenson (1938), the mean diameter of 500 cells can be measured and recorded in under one hour. Furthermore, the apparatus used is that found in all laboratories. It may be set up in four minutes.

Method and Material

The film is fixed with Leishman's stain for 1 min., rinsed with distilled water, and superstained with Field's eosin (Field, 1941) for 5 seconds. It is then dried in the air.

A "Pointolite" or similar lamp is used as a source of illumination. Any simple monocular microscope capable of being used in the horizontal position and fitted with a mechanical stage and oil immersion lens is used to project the image of the cells on to a series of rings drawn in indian ink on a white card mounted in a flat wooden base (Plate II*a*). The rings are drawn with a fine-pointed compass with intervals of 1/2 mm. The range required is from 4.0 to 12 mm. A line 50 mm. long is also drawn on the card for calibration purposes.

The apparatus used should be set up in a darkened room on a horizontal bench which should be as flat as possible.

The mirror is removed from the microscope, which is placed in a horizontal position. The mirror is then held in a retort stand so as to reflect the light passing through the microscope on to the measuring rings laid on the bench (Plate II*b*). The oil immersion lens is now used to adjust the magnification to $\times 1{,}000$ by projecting the image of the side of a small square in the central ruled area of a thin Thoma-Zeiss haemocytometer (50 μ) on to the prepared card on its board. The haemocytometer grid should have been darkened by smearing on a little Leishman stain and allowing it to dry. The card on its board is adjusted on the bench so that the image of the square lies on the 50 mm. line. By altering the distance of the mirror from the object lens of the microscope the side of one square of the image is made to coincide with the 50 mm. line. The magnification is then $\times 1{,}000$.

The stained blood film is now substituted for the haemocytometer and the image of the red cells is sharply focused on the card. By moving the card the cell diameters are measured by finding the ring which accurately contains the image of the cell. If the cell measured is not round, the ring chosen is that which is of such a size that the part outside the ring would fit into the gap left inside the ring.

In this way the diameters of as many cells as are required can be measured and recorded. The mean diameter and the standard deviation are calculated in the usual way.

Comment

The film should be as thin as possible and the part chosen for measurement should be where the red cells are not touching each other.

It is not necessary to measure 500 cells in every case, for, as Mogenson points out, the reduction

* From the John Burford Carlill Laboratories.

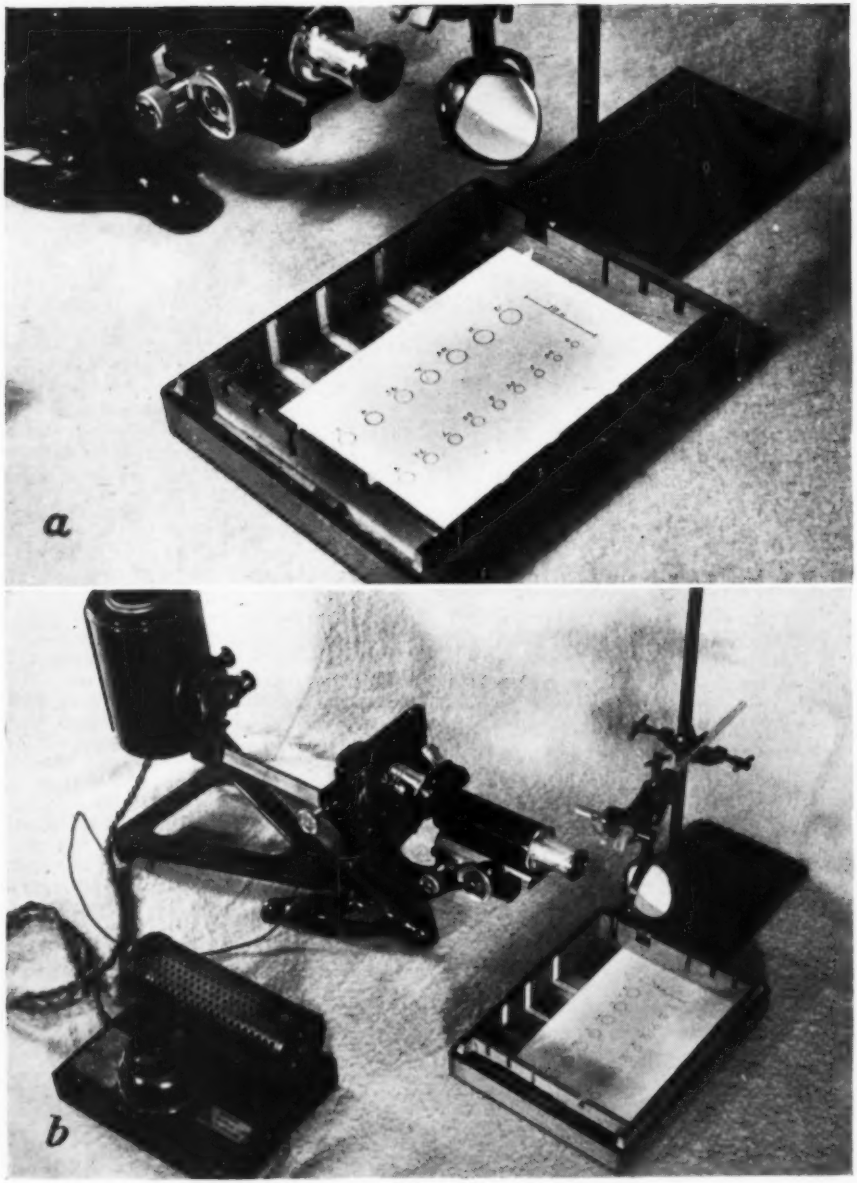

PLATE II.—(*a*) The card with the measured circles and 50 mm. line. (*b*) General view of the apparatus as set up.

o' the standard error of the mean diameter ob- t ined by increasing the number of cells examined f om 200 to 500 is 7.2 per cent if the standard d viation is normal (up to 0.48) and only 19.2 per c nt if the standard deviation is increased to 0.8.

The magnification should not be much increased a ove ×1,000, for the distinctness of the image d creases the further the light has to travel. The r solving power of the microscope also puts a limit t the magnification that can usefully be employed.

Summary

A simple method of obtaining Price-Jones curves quickly and economically is described.

Our thanks are due to Dr. Hansell, of the Westminster Hospital, Department of Medical Photography, for the two illustrations.

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J. clin. Path. (1948), **1**, 80.

THE INHIBITION OF ACID PHOSPHATASES BY FORMALDEHYDE AND ITS CLINICAL APPLICATION FOR THE DETERMINATION OF SERUM ACID PHOSPHATASES

BY

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AND

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(RECEIVED FOR PUBLICATION, DECEMBER 16, 1947)

Introduction

The presence of considerable amounts of an active phosphatase with *p*H optimum about 5, normally present in human prostate, was first described by Kutscher and Wolbergs (1935). Many other tissues and fluids of the body contain acid phosphatases in variable amounts, but these are small in comparison with those found in the prostate and seminal fluid.

Normal plasma and serum contain small amounts (1 to 4 units) of acid phosphatase. It was first observed by Gutman and Gutman (1938) that the concentration of the enzyme increases in the sera of patients with metastatic carcinoma of the prostate, presumably due to neoplastic invasion of lymph or blood channels, with the escape of the enzyme into the circulatory fluids. In many instances the increases are so great as to be clearly diagnostic ; but in some cases there are only slight increases such as are occasionally found in other diseases. In such cases, therefore, a method for measuring the prostatic fraction of acid phosphatase is greatly needed.

On account of the marked susceptibility of the prostatic phosphatase to inactivation by certain narcotics, including alcohols, which was first reported by Kutscher and Wörner (1936), Herbert (1944, 1945, 1946) was able to devise a method for the specific estimation of prostatic phosphatase in serum. The basis of Herbert's method is that incubation of serum or plasma with (two-fifths volume) absolute alcohol at room temperature for 30 minutes completely destroys the prostate enzyme fraction but hardly affects the normal plasma acid phosphatase.

During the course of a careful study of this method we have tried the effect of alcohol incubation on serum acid phosphatase in a great number of cases of normal and non-prostate diseased persons. We have found that, while in many cases the serum acid phosphatase is not significantly affected by this alcohol treatment, yet in a good number of cases a marked inhibition is observed, for example in certain types of liver disorders and other diseases. In other cases the alcohol treatment leads to an actual, and unexplained, increase in the total acid phosphatase (cf. Appendix).

This directed our attention to the possibility that this lability of the prostatic enzyme to the destructive action of alcohol may be shared by other tissue phosphatases as well. It has been found that while many tissue acid phosphatases, including those of the adrenals, intestines, liver, spleen, etc., are not affected by alcohol incubation, the red cells, bile, and kidney enzymes are, on the other hand, seriously inhibited.

The red blood corpuscles are second to the prostate in regard to their high content of acid phosphatase, and contain nearly one hundred times the amount present in plasma. King and others (1945) found the acid phosphatase of prostate and red cells to be similar in many respects, including easy destruction by alcohol.

Thus, for applying Herbert's technique, the serum should be strictly free from any trace of haemolysis. This introduces great difficulty as it has been found in practice that the majority of samples of blood taken in the wards for analysis show variable degrees of haemolysis. Even when blood is taken with great care, unless the plasma is

separated as soon as possible, there is always the possibility of escape of the enzyme from the red cells into the plasma, even without detectable haemolysis. This has been found especially in cases when the corpuscles are abnormally rich in acid phosphatase. Many samples sent to the laboratory showing apparent haemolysis have to be reported "unfit for acid phosphatase determination."

For the above reasons it is felt that there is a distinct need for a more reliable method, sufficiently simple to be adopted in routine work, giving results of definite diagnostic values in prostatic cases, and not influenced by the corpuscular or other body tissue enzymes other than those of the prostate.

It has been found, after trying a series of organic substances belonging to many different groups, that formaldehyde answers many of these requirements. Under our experimental conditions, formaldehyde destroys completely the red-cell enzyme while not affecting the prostate enzyme to any appreciable degree. Other tissue acid phosphatases are variably inhibited by formaldehyde, but none is found to be so sensitive to this reagent as the corpuscular enzyme.

The normal serum or plasma acid phosphatases are also seriously damaged by formaldehyde. Thus, by applying a very simple formaldehyde technique, we can always get the prostatic fraction of serum acid phosphatase free, or nearly free, from the other interfering acid phosphatases.

Methods

The King and Armstrong (1934) phenyl phosphate method, as modified by Gutman and Gutman (1938, 1941) for the estimation of serum acid phosphatase, has been followed in principle. Certain modifications are introduced to suit either Herbert's technique or the proposed formaldehyde procedure, both of which are described here in detail.

Reagents

Acid citrate buffer.—*p*H 4.9. Disodium citrate, 21.25 g., dissolved in 1 l. distilled water containing 100 ml. N/10 HCl. The solution should be preserved with 5 ml. chloroform and kept in the cold.

Substrate.—M/50 disodium phenyl phosphate*: 2.18 g. dissolved in 500 ml. distilled water. The solution should be brought quickly to the boil to destroy any organisms, cooled immediately, and preserved with 5 ml. chloroform and kept in the cold.

Folin-Ciocalteu phenol reagent (1927).—Diluted 1 in 3.

25 per cent sodium carbonate (anhydrous) (w/v).—This solution should be kept in a warm place.

Standard-phenol-and-reagent. — (1 mg. phenol/100 ml.) 5 ml. of stock standard phenol (100 mg./100 ml.) is accurately measured into a 500-ml. volumetric flask; 100 ml. of dilute (1:3) Folin-Ciocalteu reagent is added, and water to the mark.

Formaldehyde reagent.—(2 per cent formaldehyde solution.) 5 ml. of neutral formaline solution (40 per cent) is pipetted into a 100-ml. measuring flask and diluted to the mark with distilled water.

Procedures

Fresh serum or plasma must be used, and examined with the visual spectroscope, if necessary, to be sure of complete absence of haemolysis whenever the alcohol procedure is to be adopted.

Total acid phosphatase.—1 ml. substrate and 1 ml. distilled water are pipetted into each of two centrifuge tubes together with 2 ml. of the buffer solution. The tubes are allowed to remain in a 37° water-bath for three minutes to allow the contents to attain the temperature of the bath. After this time 0.2 ml. of the serum is added to one of the tubes and the enzyme hydrolysis allowed to proceed for exactly one hour, at the end of which time 1.8 ml. of Folin and Ciocalteu's phenol reagent are added to each tube and 0.2 ml. of serum to the second tube. The two tubes are shaken and centrifuged, and 4 ml. of the supernatant pipetted into two test tubes. After the addition of 1 ml. of 25 per cent sodium carbonate solution, the tubes are replaced in the water-bath for fifteen minutes to allow the colours to develop. The colour intensity is compared with that of a standard solution of phenol (i.e., 4 ml. of standard-phenol-and-reagent and 1 ml. of sodium carbonate) which has been similarly treated. A photo-electric colorimeter is used for this purpose, setting its zero with water, or, better, a blank of 3 ml. water, 1 ml. phenol reagent, and 1 ml. sodium carbonate solution (without serum), and using a red filter. The difference between the incubated and non-incubated samples gives the phenol formed by hydrolysis. A unit of acid phosphatase is defined as the amount of enzyme which will liberate 1 mg. phenol per 100 ml. serum or plasma per hour from M/200 disodium phenyl phosphate substrate at a *p*H near 5 at 37° C. (If the colours are too deep for reading in the available colorimeter, the coloured solutions (test, control, blank, and standard) should be diluted to 10 ml. with water.)

Calculation:

$$\left(\frac{T}{S} \times 0.04 \times \frac{6}{4} \times \frac{100, \text{ for } 0.2 \text{ test}}{}\right) - \left(\frac{T}{S} \times 0.04 \times \frac{6}{4} \times \frac{100, \text{ for } 2 \text{ control}}{}\right)$$

$$= \left(\frac{T}{S} \times 30, \text{ for test}\right) - \left(\frac{T}{S} \times 30, \text{ for control}\right) = \text{units phosphatase/100 ml.;}$$

where T = reading of test; S = reading of standard; 0.04 = mg. phenol in the 4 ml. of standard-phenol-and-reagent; 6 = the vol. of 1 ml. substrate + 1 ml.

* Commercial specimens of disodium phenyl phosphate may contain traces of free phenol, which add appreciably to the blank colour in the phosphatase method. Any free phenol is easily got rid of by washing the solid disodium phenyl phosphate with ether, after which it should be dried in the air.

water + 2 ml. buffer + 0.2 ml. serum + 1.8 ml. Folin-Ciocalteu; 4 = the vol. of protein-free supernatant taken; and 0.2 = the vol. of serum.

Alcohol inactivation.—To 1 ml. of serum or plasma, 0.4 ml. of absolute ethyl alcohol is added. After shaking, the tube is stoppered and the mixture allowed to stand for half an hour at room temperature. Water (0.6 ml.) is added, and a sample of 0.4 ml. of the opalescent mixture (corresponding to 0.2 ml. of serum or plasma) is added to the warm, previously mixed, 2 ml. buffer + 1.0 ml. substrate and 0.8 ml. water to make a final volume of 4.2 ml. buffer–substrate–enzyme mixture. The procedure is then followed exactly as above for total acid phosphatase.

Formaldehyde technique.—The procedure is exactly as described above under "total acid phosphatase" except that, in this case, 1 ml. formaldehyde reagent is added instead of the 1 ml. distilled water to the mixture of 2 ml. buffer and 1 ml. substrate solutions. All the following details are then the same.

Experimental

The Relative Content of "Acid" and "Alkaline" Phosphatases in Some Human Tissues

Since we are mainly concerned with the serum acid phosphatases, we thought it necessary to examine the various body tissues which are believed to be responsible for the variation of this enzyme in plasma in health and disease.

Although a good deal of work described in the literature has been carried out on various animal tissues in this connexion, very little has actually been done on human material. Normal adult tissues, without any apparent disease, were obtained from autopsy within 24 hours of death. After being freed from fat and other adhering extraneous tissue, they were carefully washed with normal saline and then with cold tap water until they were as free as possible from any blood cells. They were then dried by filter paper, weighed and ground with sand, and mixed intimately in a mortar with an equal weight of normal saline solution and 10 per cent (by volume) of a mixture of equal parts of toluene and ethyl acetate. After having been left to autolyse at room temperature for two or three days with occasional shaking, the mixtures were centrifuged and the supernatant liquids filtered clear through a Whatman No. 1 folded filter paper.

Tissue extracts obtained in this way are always clear and free from any colour of blood, and their enzyme activity corresponds to that present in the original wet tissue. They keep their activity unchanged for several weeks if kept in the ice chest. They should be diluted ten to twenty times, or even more in certain cases, with normal saline just before their activity is determined. In the case of the red cells, the freshly drawn blood was directly centrifuged, the plasma separated, and the red cells washed twice with normal saline by spinning and decantation. 1 ml. of washed red cells was lysed in 9 ml. water and the solution centrifuged. The clear supernatant was used directly for determining its enzyme activity. The prostate gland was decapsulated, freed from adhering tissue, cut into fine pieces, and ground with sand in a mortar with five times its weight of normal saline solution. A few drops of toluene were added but no ethyl acetate. The mixture was left at room temperature to autolyse for two or three days with occasional gentle rotating. (Vigorous shaking should be avoided as it may inactivate the enzyme.) The mixture was then filtered through filter paper. It was highly diluted (e.g.—1,000 to 5,000 times) before determining its enzyme activity.

TABLE I

ALKALINE AND ACID PHOSPHATASE OF HUMAN TISSUES

Tissue	No. of specimens examined	mg. phenol liberated/hr./1 g. wet weight tissue				Ratio alkaline/acid
		Alkaline phosphatase		Acid phosphatase		
		Range	Average	Range	Average	
Adrenal glands	6	25–38	30	2–5.5	3.0	10
Bile from gall-bladder	5	10–23	16	0.3–7.0	2.5	6.2
Intestinal tissue	5	49–72	53	2–3	2.7	20
Kidney	6	12–24	16	6.5–18	10	1.6
Liver	6	12–37	14	7–13	9.7	1.4
Pancreas	5	0–6	3	3–11	5.7	0.5
Prostate	10	0.1–1.2	0.5	2,000–10,000	4,000	0.0002
Red cells	20	0.6–2.5	1.5	8–25	15	0.1
Spleen	5	10–17	14	4.5–8.5	6.7	2.0
Thyroid	4	2–4	3	1.5–3	2.0	1.5

The phosphatase activity of these preparations was determined at two hydrogen ion concentrations; namely, *p*H 5 and *p*H 10, using the above-described acid citrate buffer for the first, and alkaline carbonate-bicarbonate buffer (Delory and King, 1945) for the latter, with disodium phenyl phosphate as substrate. For the sake of comparative study of the relative activity of the two enzymes at these two *p*H values, the buffer–substrate–enzyme mixtures were incubated for 60 minutes at 37° C. The phenol liberated at the end of that time was taken as an index of the enzyme activity at the corresponding *p*H.

Thus Table I gives the results expressed in mg. phenol liberated per 1 g. wet tissue per hour at the two given *p*H values. (No Mg was added.)

Table I does not include the bone phosphatases, which, in the normal adult, are not expected to be of much activity. The intestines are the richest human source of alkaline phosphatase, followed by the suprarenals. The human kidney seems to be very poor in alkaline phosphatase compared with other animals' kidneys, while the human liver appears to contain much more alkaline enzyme than that of other animals.

As for the acid phosphatases, the prostate is unquestionably the richest source in the human. The only other animal in which it occurs in large amounts is the monkey and then only in the caudal lobe of the prostate, as shown by Gutman and Gutman (1939). The second tissue that is rich in acid enzyme is the red cell, although the difference is very great. Red cell phosphatase varies enormously in different individuals, for no known reason, but generally speaking it has been found to diminish in activity with age.

The human kidney, liver, pancreas, and spleen contain appreciable amounts of acid phosphatase.

Effect of Alcohol Incubation and Formaldehyde Treatment on Different Tissue Acid Phosphatases

The tissue extracts described above were subjected to both alcohol incubation and formaldehyde treatment in the same way as described for serum. Table II shows the result of several experiments carried out in this connexion on different tissue extracts.

From Table II the inhibitory action of alcohol on prostate, bile, kidney, and red cells is evident.

The failure to achieve 100 per cent inactivation of the prostate enzyme by alcohol incubation can be explained by the fact that we were working with enzyme solutions in normal saline. Herbert showed that certain conditions must be fulfilled in order to get complete inactivation of the prostate enzyme solution with alcohol incubation; for example, the presence of a sufficient bicarbonate concentration and other serum salts of weak acids and strong bases is essential.

TABLE II

EFFECT OF ALCOHOL AND OF FORMALDEHYDE ON ACID PHOSPHATASES

Tissue preparation	Acid phosphatase units/ 100 ml.*	Percentage inhibition	
		After alcohol	With formaldehyde
Adrenals ..	21–64	0	12–25
Bile ..	1.5–34	80–100	20–50
Intestines ..	35–71	0	40–50
Kidneys ..	37–153	17–62	24–50
Liver ..	35–92	0	30–60
Pancreas ..	30–60	0	15–40
Prostate ..	25–300	40–70	0
Red cells ..	9–120	40–55	100
Spleen ..	17–127	0	44–52
Thyroid ..	20–75	0	25–40

* These figures represent the enzyme activities of extracts of tissues at various dilutions prepared to give a convenient concentration of phosphatase for accurate measurement. They do not represent the comparative enzyme contents of the tissues themselves.

The destruction of the bile acid phosphatase with alcohol is especially striking, since that of the liver is not affected. It might be possible that this inhibition is indirectly due to certain other bile constituents affected by the alcohol incubation. Until both the liver and the bile enzymes are prepared in a purer state, we cannot decide if there is a real difference between them.

Formaldehyde inhibits the different tissue enzymes to variable extents (20 to 60 per cent), but its 100 per cent inhibitory action on red cells is remarkable; likewise its complete lack of any effect on the prostatic phosphatase.

The difference between the red-cell enzyme on the one hand, and the liver and spleen enzymes on the other, which was first described by Davies (1934), is further shown here in regard to their behaviour towards alcohol and formaldehyde.

Effect of Alcohol Incubation and Formaldehyde Treatment on Normal Plasma and on Prostate and Red Cell Solutions in Plasma

The following illustrations of the effect of alcohol incubation and formaldehyde treatment on normal plasmas alone, and on those to which prostate extracts and red-cell solutions have been added, form the basis for the clinical application of these methods.

It has been shown by Herbert that in the case of alcohol incubation the behaviour of the prostate enzyme solution in normal saline differs from that in serum or plasma. It is more strongly inhibited when present in the latter state than when it is merely in saline solution. Thus the question arises as to whether the prostate and red-cell enzyme solutions in water will behave differently to those in serum or plasma with regard to the formaldehyde treatment. The answer is found in Table III, from which it is seen that in plasma or serum media the formaldehyde still exhibits its strong inhibitory action on the red-cell enzyme while not affecting at all the prostate phosphatase.

Normal plasma phosphatases are sensitive to formaldehyde but resistant to alcohol. The alcohol incubation seems to destroy totally the prostate enzyme in serum or plasma medium, while the red-cell enzyme is only partly destroyed.

The formaldehyde, however, provides a quantitative means for estimating the prostate enzyme in the presence of the red cell. This is clearly shown in Table IV, in which two solutions, one of prostate extract and the other of red cells, both of known enzyme activity, are mixed together in different proportions and subjected to formaldehyde treatment. The residual values after formaldehyde treatment always agree closely with those corresponding to the amount of prostate enzyme added. Table IV shows that a quantitative determination

TABLE III

EFFECT OF ALCOHOL AND FORMALDEHYDE ON PLASMA PHOSPHATASE ENRICHED BY THE ADDITION OF PROSTATE AND RED-CELL PHOSPHATASES

	Acid phosphatase units/100 ml.	After alcohol incubation	After formaldehyde treatment
1. Normal plasma	4.5	4.0	1.1
,, ,, + prostate ext.	48.6	6.3	45.0
,, ,, + prostate ext.	73.5	3.7	69.7
,, ,, + red cells soln.	45.7	17.3	1.5
2. Normal plasma	6.0	5.2	1.5
,, ,, + prostate ext.	100.5	6.7	95.2
,, ,, + red cells soln.	37.5	24.0	3.7
,, ,, + red cells soln.	66.0	15.0	3.2
3. Normal serum	3.0	4.0	1.5
,, ,, + prostate ext.	48.0	3.0	47.2
,, ,, + red cells soln.	24.0	13.2	1.5
4. Plasma	3.5	4.6	1.5
,, + prostate ext. ..	49.5	4.5	47.5
,, + red cells ..	31.5	10.5	1.5

TABLE IV

AGREEMENT BETWEEN THE CALCULATED AND DETERMINED ACID PHOSPHATASE CONTENTS OF PROSTATE–RED-CELL MIXTURES

	Units/100 ml. solution		Calculated units of prostate enzyme in mixture
	Without formaldehyde	With formaldehyde	
Solution (A) prostate ext.	93	93	
Solution (B) blood cells	30	0	
Mixture of (A) + (B)			
1(A) + 1(B)	64.5	48.0	46.5
1(A) + 2(B)	51.5	31.5	31.0
2(A) + 1(B)	73.0	62.0	62.0

TABLE V

INHIBITING EFFECT OF VARIOUS CONCENTRATIONS OF FORMALDEHYDE ON TISSUE ACID PHOSPHATASES

	Units/100 ml. before treatment	Units/100 ml. after treatment with formaldehyde			
		1%	0.5%	0.25%	0.1%
Normal serum	4.3	1.5	1.5	3.3	3.7
Prostate extract	150.0	140.0	148.5	150.0	150.0
Normal serum + prostate	45.5	39.8	42.5	43.5	44.0
Red cells in water	40.0	0	0	0	0
,, ,, ,, ,, ...	140.0	0	0	12.0	15.0
Normal serum + red cells	24.0	1.5	1.5	3.0	3.5
Adrenals	21.0	16.5	—	—	21.0
Kidney extract	38.6	20.5	21.5	22.6	24.6
Liver ,, ...	35.5	18.7	21.4	22.0	23.6
Pancreas ,, ...	13.5	11.2	11.2	—	11.2
Spleen ,, ...	56.2	24.1	26.8	27.5	—

of prostate and red-cell enzymes, when present in a mixture, can be easily effected.

Effect of Various Concentrations of Formaldehyde on Different Acid Phosphatases

The concentration (final) of formaldehyde, so far used in all the previous experiments, and which is suggested for use in routine work, is 0.5 per cent in the enzyme–substrate–buffer mixture.

This concentration has been found experimentally to be the maximum that can be used without affecting the prostate enzyme, while producing its maximum inhibition of the serum acid phosphatases. Table V shows the effect of various concentrations of formaldehyde. The extreme sensitivity of the red-cell enzyme to formaldehyde is apparent. As little as 0.1 per cent of this reagent completely destroys small amounts of the enzyme, while inhibiting nearly 90 per cent red-cell preparations of high phosphatase activity (for example, 140 units/100 ml.). On the other hand solutions of formaldehyde stronger than 0.5 per cent affect the prostate enzyme. The plasma and serum acid phosphatases seem not to be inhibited beyond certain limits even with increase of formaldehyde concentration. The 0.5 per cent concentration of formaldehyde seems, therefore, to be just suitable for the purpose.

Clinical Application

The alcohol and formaldehyde techniques have been applied to a variety of plasmas and sera from different types of disease, covering 67 prostate cases, 24 cases of other tissue cancers, 15 cases of bone disease, 38 cases of liver disease, 16 cases of kidney disease, and 56 cases of other miscellaneous diseases.

The majority of these samples were subjected to both formaldehyde and alcohol incubation procedures, except in some instances where there was either shortage of material or where the samples showed slight signs of haemolysis. The majority of samples were collected from the Hammersmith Hospital, others from other London hospitals, and the sera and plasmas, which were separated as soon as the blood was received, were kept in the ice chest until the tests could be carried out (usually without delay).

Results and Discussion

A synopsis of the results of the clinical application of both alcohol incubation and the formaldehyde method to pathological sera and plasma is given in Table VI and the results appear in full in the appendix.

For convenience the "total acid phosphatase" figures are classified into two main groups: "normal values" (those up to 5 units); and "abnormal values" (more than 5 units per 100 ml.).

TABLE VI

SUMMARY OF ACID PHOSPHATASE RESULTS

(Total acid phosphatase units in roman type, formaldehyde-stable in italics)

Diagnosis	Number of cases with acid phosphatase in the range												
	0 to 1.5	1.6 to 3	3.1 to 5	5.1 to 7	7.1 to 10	10.1 to 15	15.2 to 20	21 to 30	31 to 40	41 to 50	51 to 70	71 to 100	>100
Carcinoma of prostate with bone metastases (22)*	—	2	4	1	1	2	4	2	—	2	—	—	4
	4	*2*	*2*	—	*3*	*2*	*2*	*1*	*1*	*1*	—	—	*4*
Carcinoma of prostate; no bone metastases demonstrable (22)	—	4	6	4	4	3	—	—	—	—	—	—	1
	5	*9*	*5*	*1*	—	*1*	—	—	—	—	—	—	*1*
Senile hypertrophy, and retention with enlarged prostate (23)	—	9	4	3	2	—	3	2	—	—	—	—	—
	8	*8*	*1*	*2*	*2*	—	*1*	*1*	—	—	—	—	—
Cancer of organs other than prostate (24)	—	3	8	5	4	4	—	—	—	—	—	—	—
	9	*12*	*3*	—	—	—	—	—	—	—	—	—	—
Paget's disease of bone (15)	—	4	5	4	1	1	—	—	—	—	—	—	—
	11	*1*	*2*	*1*	—	—	—	—	—	—	—	—	—
Obstructive jaundice (18)	1	2	2	7	4	1	1	—	—	—	—	—	—
	2	*7*	*6*	*2*	*1*	—	—	—	—	—	—	—	—
Other liver disease (hepatitis, cirrhosis, etc.) (20)	—	7	5	1	4	1	1	1	—	—	—	—	—
	7	*6*	*5*	*2*	—	—	—	—	—	—	—	—	—
Nephritis (16)	2	7	1	3	1	2	—	—	—	—	—	—	—
	7	*4*	*3*	*2*	—	—	—	—	—	—	—	—	—
Miscellaneous (56)	5	18	16	6	3	3	3	2	—	—	—	—	—
	29	*20*	*3*	*4*	—	—	—	—	—	—	—	—	—

* Number of cases.

Prostate Cases

The prostate cases examined, although not great in number, furnish a useful conclusion as to the successful clinical application of the formaldehyde technique. These cases are as follows:

Cases of carcinoma of prostate with bone metastases.—These are 22 in number; 16 of them gave abnormal values (>5) for the total acid phosphatase (73 per cent). Compared with the latest published results, Herbert (1946) found that about 83 per cent of 35 cases of prostatic cancer showing bone secondaries gave acid phosphatase values above 5, while Woodard and Dean (1947) found 72 per cent of 71 cases examined to give abnormal values.

Considering the formaldehyde-stable fraction in cases examined in the present work, it is noted that 16 cases out of the 22 (73 per cent) gave values above 3 units, while 14 of the latter gave values above 5 units. This means that if we adopt the figure 3 as the maximum permissible normal value for the formaldehyde-stable serum acid phosphatase (see below), we still get the same percentage of abnormal values.

The formaldehyde-stable fraction in most abnormal cases coincides fairly well with the alcohol-labile fraction, that is, with the difference between the total acid phosphatase and the alcohol-stable fraction (except in a few cases where the alcohol-stable fractions were unexpectedly high). If the two figures (after both alcohol incubation and formaldehyde treatment) be added together, values very near to the original total acid phosphatase are obtained (see Appendix).

The formaldehyde procedure provides a more reliable means of following the progress of cases under oestrogenic treatment than does the total acid phosphatase. High "total" values have shown a pronounced tendency to fluctuation, whereas the formaldehyde-stable figures steadily decreased during the course of favourable responses to treatment.

Cases of carcinoma of prostate without bone metastases (as shown by histological and radiological examination).—These are 22 in number. In 12 of them (55 per cent) the total acid phosphatase figures are above normal (i.e., >5); while with formaldehyde only 7 (32 per cent) gave values above 3 units and only 3 (14 per cent) gave values above 5 units. In one case only was the acid phosphatase very high (>100): repeated radiological examination failed to reveal any evidence of bone secondaries, until, in the latest film, an apparent abnormality was noted in one of the lumbar vertebrae.

Other prostate cases.—These comprise 23 cases of adenoma, acute retention with enlarged prostate, and senile hypertrophy. Most of these cases were examined radiographically and no evidence of bone lesion was found. The prostate growths were found histologically to be benign.

Ten cases (45 per cent) showed abnormal total acid phosphatase values while 7 (32 per cent) gave formaldehyde-stable figures above 3 units, and 6 (27 per cent) gave values above 5 units.

Non-Frostatic Cases

Cancer of various organs.—In this group figures are given for 24 cases of cancer of different organs other than the prostate. In some of these there were secondaries in the bones, and the akaline phosphatase in one case is very high (85 units). In many of the cases there is a tendency to an increased total acid phosphatase value, not necessarily, however, paralleled by an increase in the akaline phosphatase: for example, the highest total acid phosphatase value (15 units obtained in a case of cancer of bone) was accompanied by an akaline phosphatase of only 23 units; while the case of carcinoma of the bronchus, invading several organs including bone, with the very high alkaline phosphatase (85 units), had only 3.4 total acid phosphatase units.

Of the 24 cases examined, 13 gave abnormal total acid phosphatase values (that is, above 5 units; about 54 per cent). With the formaldehyde technique none gave values above 5 units, and only 3 cases (about 12 per cent) gave values above 3, the highest of them being only 4 units.

Paget's disease of bone.—Fifteen cases of bone disease were studied. All of them were Paget's. Five are moderately high and one high. In Paget's disease there is no parallelism between the total acid phosphatase and the increased alkaline phosphatase values.

It is known that the phosphatase of bone is very sensitive to an acid *p*H, and one would not, therefore, expect any activity on the acid side of neutrality from bone phosphatase preparations, however high their activity might be on the alkaline side. Consequently, any increase in acid phosphatase in bone disease should be considered of non-osseous origin. In secondary carcinoma of bone, with the primary growth in organs other than the prostate, there might be a tendency, however, to high total acid phosphatase values. This was found to be the case in two sera examined. But formaldehyde treatment reduced all values obtained in bone disease to a range from 0 to about 3 (except in one Paget's, where it was 5.5).

Some authors are of the opinion that an extremely high serum akaline phosphatase derived from bone may show its activity slightly at the acid reaction. But formaldehyde will exclude this completely, as the alkaline bone enzyme is very sensitive to this reagent. In the one Paget's case with an alkaline phosphatase of 140, and a formaldehyde resistant phosphatase of 5.5 (out of a total acid phosphatase of 7.5), the 5.5 value cannot be taken as a mere reflection of the very high alkaline phosphatase of bone origin. Such elevated phosphatases must be of non-osseous origin.

The possibility of the local production of acid phosphatases in tumours and abnormal active growths should not be neglected. Gutman and others (1936) observed that, in addition to alkaline phosphatase, acid phosphatase was markedly increased at the site of osteoplastic metatases secondary to carcinoma of the prostate gland. This was confirmed by Barringer and Woodard (1938). This acid phosphatase may be different from that of the prostate since it seems to be formaldehyde labile in most cases, as can be judged from the high total acid phosphatase levels, compared with the comparatively small formaldehyde-stable values, in cases of carcinoma of the prostate with bone metastases during treatment with oestrogens.

Liver disease.—The most striking incidence of high values of non-prostatic acid phosphatases occurs in liver diseases. 21 cases out of the 38 studied gave raised total values above 5 units. As has been previously pointed out, the bile acid phosphatase, which might account for some of the high values found in jaundice and other liver disease, is completely destroyed by the alcohol incubation and partially by formaldehyde. On the other hand the liver acid phosphatase is not attacked by alcohol and only partly by formaldehyde. The results of alcohol incubation and formaldehyde treatment, shown in the table, suggest that the abnormal amount of acid phosphatase present is derived both from the liver and from the bile, since it is only partly destroyed by both alcohol and formaldehyde in most cases.

Although formaldehyde treatment does not furnish a complete differentiation in cases of liver disease which show a tendency towards elevated formaldehyde-stable values (reaching 7.5 in 1 case), nevertheless it is still the most useful indicator we have (although some other means of sharper differentiation may eventually be found).

Miscellaneous diseases.—The result of applying the inactivation methods to the serum acid phosphatases in a variety of other diseases indicates that there is a tendency for an increased acid phosphatase in some kidney cases. The alcohol incubation method affected quite a number of them. In a total of 53 cases (all free from haemolysis) subjected to the alcohol incubation method, 13 showed reduction of more than 1 unit.

As regards the formaldehyde treatment, it is noted that out of the total of 72 cases examined by this technique, 60 (i.e., 84 per cent) of them gave formaldehyde-resistant values between 0 and 3 units, while 6 (8 per cent) gave figures of 3 to 5, and 6 above 5 units.

It is striking that in some cases, for example, of anaemia, there is a small total acid phosphatase which is totally resistant to formaldehyde. This is not necessarily to be taken as of prostatic origin, for it occurs in both male and female cases. There may be present in some tissues not yet shown an acid phosphatase which is similar to the prostate enzyme in its stability towards formaldehyde, but which is not destroyed by alcohol.

Conclusion

The value of 5 units of total acid phosphatase, arbitrarily agreed upon by several workers as the maximum (normal figure) for non-prostate cases, has been exceeded in a good number of non-prostate cases. It is desirable to have a method giving a more restricted range in non-prostatic cases while not affecting the prostatic acid phosphatase. This has been satisfactorily found in the proposed formaldehyde technique.

Although the number of cases examined (216 in all) is not great, yet we believe we have sufficient data to support the following suggested limits for the formaldehyde-stable acid phosphatase:

Normal values: from 0–3 units per 100 ml. serum or plasma.

"Suspicious": from 3.1–5 units per 100 ml. serum or plasma.

Prostatic: above 5 units per 100 ml. serum or plasma.

In most cases the simple formaldehyde treatment will serve to distinguish between the high acid phosphatase of prostatic origin and those accompanying other conditions. In a few cases this will not suffice, and Miss Herbert's technique of alcohol treatment should then be employed. But experience seems to show that certain liver cases (and possibly some cases of bone disease) are the only instances where confusion with prostatic disease is likely to arise, and here an alkaline phosphatase, together with other characteristic chemical and clinical tests, should resolve any ambiguity

In addition to being useful for the study of special and difficult cases, formaldehyde and alcohol have a definite utility, along with other inhibitors and activators, in attempts to differentiate the several acid phosphatases of the different tissues of the body. We have evidence, for instance, of at least 2 acid phosphatases in most tissues and of there only being one in the prostate.

Summary

1. A study of the relative content of the acid and alkaline phosphatases of some human tissues has been made.

2. The effect of alcohol incubation was tried on these tissue acid phosphatases, and it has been shown that while those of the adrenals, intestine, liver, pancreas, spleen, and thyroid are not affected, those of the bile, kidney, red cells, and prostate, on the other hand, are seriously inhibited.

3. The effect of formaldehyde on the acid phosphatases of prostate and other tissues, including the red cells, was studied under certain conditions. Its complete destructive action on the red-cell enzyme, and its lack of any effect on the prostate enzyme under the same conditions, are reported. The other tissue acid phosphatases are variably inhibited, but none is similar to the red-cell enzyme in being completely inactivated.

4. The introduction of a formaldehyde technique in routine determinations of the serum or plasma acid phosphatase is suggested, and a method described.

5. The alcohol incubation method and the formaldehyde technique were applied to 216 pathological sera and plasmas, including prostate cases and other cases of a wide variety of diseases. The results are discussed, and limits for the formaldehyde-stable fraction of serum acid phosphatase for what should probably be considered non-prostate, are suggested.

6. A high acid phosphatase after formaldehyde treatment (above 5 units) strongly suggests a prostatic origin (values >3 are "suspicious"), and in most cases it gives useful information for distinguishing between raised values due to the presence of prostatic phosphatase, and those of different origin. An alcohol incubation test gives further confirmatory evidence in doubtful cases.

7. The formaldehyde technique permits the use of haemolysed sera or plasmas which are otherwise unfit for acid phosphatase determination.

We are indebted to Dr. E. P. Sharpey-Schafer, Dr. Jean Watkinson, Dr. G. A. Harrison, Dr. N. F. Maclagan, Dr. G. Discombe, Dr. F. T. Prunty, Prof. J. R. Marrack, and Dr. J. Goodie for samples provided.

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APPENDIX

PLASMA ACID PHOSPHATASE (TOTAL, ALCOHOL-STABLE, AND FORMALDEHYDE-STABLE) IN PATHOLOGICAL SERA

Case	Acid phosphatase (units/100 ml.) Total	Alcohol-stable	Formaldehyde stable	Alkaline phosphatase (K.A. units/100 ml.)	Notes
PROSTATE CASES (67) Carcinoma of prostate with bone metastases (22)					
1					
14.1.47	—	—	—	—	Admitted to hospital. Very ill
20.1.47	119	19	119	127	10 mg. oestradiol (i.m.)+20 mg. stilboestrol (0.) daily for 8 days
29.1.47	55	5.8	49	120	Little improvement
4.2.47	39	—	33	128	40 mg. stilboestrol daily for 10 weeks
26.2.47	17	—	11	86	Improvement
3.3.47	17	—	12	76	
10.3.47	17	—	11	142	
14.3.47	23	—	6.0	180	
17.3.47	13	—	0	150	
19.3.47	9.0	—	1.5	118	Intermittent oedema
23.3.47	9.0	—	4.5	105	
26.3.47	7.5	—	3.0	58	Condition eased
2.4.47	8.2	—	3.7	65	
9.4.47	3.0	—	1.8	44	
PROSTATE CASES (67) (*continued*)					
1 (*contd.*). Carcinoma of prostate with bone metastases (22) (*contd.*).					
17.4.47	22	—	6.6	48	Liver enlarged
24.4.47	14	—	7.3	43	200 mg. stilboestrol orally daily for 30 weeks
1.5.47	17	22	5.1	40	
8.5.47	21	23	5.1	43	
12.5.47	8.2	8.0	2.1	39	
15.5.47	9.2	10	3.7	38	
22.5.47	12	—	3.5	36	
29.5.47	13	—	5.0	36	
5.6.47	11	—	3.0	45	
12.7.47	4.7	3.7	2.2	33	
23.8.47	—	—	—	—	Discharged on 200 mg. stilboestrol daily. Greatly improved, able to walk
29.9.47	9.5	11	0	9.6	Liver not palpable
29.10.47	6.0	6.0	3.0	13	Condition still good

Case	Acid phosphatase (units/100 ml.) Total	Alcohol-stable	Formal-dehyde-stable	Alkaline phos-phatase (K.A. units/ 100 ml.)	Notes
PROSTATE CASES (67) (*continued*)					
Carcinoma of prostate with bone metastases (22) (*continued*)					
2	145	2.7	145	—	
3	117	3.7	113	—	
4	113	0.5	112	—	
5	50	5.2	47	42	
	21	—	17	81	
	2.5	—	2	18	
6	45	—	38	—	Stilboestrol treatment
	10	4.5	4.2	24	
7	30	1.3	28	—	
8	21	0.8	20	—	
9	20	3.4	18	—	
10	19	—	14	12	
11	16	7.3	8.7	32	
12	11	3.7	8.2	18	
	18	—	9	11	
13	18	1.8	15	—	
14	11	—	4.5	—	
15	9	0.5	9	—	
16	6.8	5.4	4.8	37	
17	4.6	4.6	1.2	—	
18	4.5	0.7	2.3	—	
	1.2	—	0.6	5.2	
19	3.8	3.8	1.5	—	
20	3.7	3.4	2.0	14	
21	3	2.1	0.5	—	
22	3	1.9	0.8	—	
Carcinoma of prostate; no bone metastases demonstrated (22)					
1	146	—	145	—	
2	14	3.0	12	15	
3	12	8.2	3.1	—	
	4.5	—	1.5	7.5	
4	11	8.8	2.5	—	
5	2.6	3.5	1.9	9	
6	9.5	—	3.7	14	
7	9	12	2.3	20	
	4.3	4.3	1.4	14	
8	7.9	—	6.8	—	Stilboestrol treatment
	3.9	—	1.5	—	
	2.5	—	1.4	—	
9	7.2	—	3.2	—	
10	6.8	5.4	4.8	16	
11	6.7	17	1.1	13	
12	6	0.7	0	—	Haemolysed
13	5.3	0.6	4.7	—	
14	4.7	—	1.3	—	
15	4.3	—	2.8	—	
16	4.2	3.3	3.0	—	
17	4.2	—	2.2	—	
18	4.0	4.5	1.8	—	
19	3.5	—	2.1	27	
20	3.0	2.0	3.0	—	
21	2.9	2.9	1.4	13	
	1.2	—	1.2	10	
22	2.9	—	0	—	
Other prostate cases (23)					
1	30	—	23	9	Senile hyperplasia
2	23	—	7.5	—	Retention
3	20	—	7.5	—	Adenoma
4	19	7.5	17	—	,,
5	17	—	4.5	23	Retention
6	10	6.2	6.9	—	,,
7	8.5	6.5	5.8	10	,,
	6.0	—	4.0	—	
8	6.6	4.6	2.7	—	,,
9	6.0	—	3.0	—	,,
10	5.8	5.8	1.3	—	,,
11	4.1	—	0	—	Adenoma
12	3.7	3.3	2.7	7.5	Senile hypertrophy
13	3.4	1.3	2.6	—	Retention
14	3.3	—	0	—	,,
15	2.8	—	0.7	7.0	Adenoma
16	2.7	0.7	2.0	23	Senile hypertrophy
17	2.7	3.0	1.8	—	,, ,,
18	2.7	1.3	0.4	—	Adenoma
19	2.4	—	0	—	,,
20	1.9	1.9	0.7	—	Retention
21	1.8	2.0	1.8	—	,,
22	1.8	1.5	0.7	—	,,
23	1.7	0.4	1.7	—	Obstruction

Case	Acid phosphatase (units/100 ml.) Total	Alcohol-stable	Formal-dehyde-stable	Alkaline phos-phatase (K.A. units/ 100 ml.)	Notes
CANCER OF VARIOUS ORGANS (24)					
1	13	—	1.6	15	Cancer of bladder
2	15	14	3.0	23	,, ,, bone
3	9.5	—	4.0	25	,, ,, ,,
4	3.6	3.0	2.7	47	,, ,, breast with metastases
5	9.0	10	2.4	50	,, ,, breast
6	2.2	3.6	2.2	—	,, ,, bronchi
7	3.4	3.7	0	85	,, ,, ,,
8	6.2	3.0	2.7	8.5	,, ,, ,,
9	2.7	2.1	1.3	5.2	,, ,, colon
10	2.3	1.8	0.4	9.0	,, ,, ,,
11	13	—	1.6	15	,, ,, lung
12	11	—	3.0	—	,, ,, ,,
13	8.3	—	1.6	17	,, ,, ,,
14	7.5	7.5	1.9	—	,, ,, ,,
15	6.0	—	2.3	45	,, ,, ,,
16	5.1	—	3.3	—	,, ,, ,,
17	4.8	3.5	0.6	11	,, ,, ,,
18	3.7	—	1.5	—	,, ,, ,,
19	5.4	3.6	1.2	30	,, ,, liver
20	4.1	4.0	1.8	33	,, ,, ,,
21	5.2	4.5	3.7	—	,, ,, pancreas
22	4.5	3.0	1.5	—	,, ,, oesophagus
23	6.4	9.3	1.1	—	,, ,, rectum
24	3.6	3.0	0	7.8	,, ,, ,,
PAGET'S DISEASE OF BONE (15)					
1	12	7.6	3.3	20	
2	7.5	8.0	5.5	140	
3	6.7	6.0	0.4	15	
4	6.7	5.5	3.4	15	
5	6.3	5.4	2.9	75	
6	5.2	5.2	1.1	81	
7	4.5	3.0	1.5	76	
8	3.4	3.0	0.4	9.1	
9	3.1	2.9	0.8	30	
10	4.1	—	0.5	62	
11	4.0	—	1.3	—	
12	2.9	2.2	1.4	20	
13	2.7	—	0	—	
14	2.3	2.5	0	25	
15	2.3	—	0	—	
LIVER DISEASE (38)					
1	9.6	—	1.2	17	Cholecystitis
2	2.5	2.0	0.2	—	,,
3	3.7	0.8	1.5	14	Cirrhosis
4	3.0	3.0	2.7	13	,,
5	2.2	0.9	0.4	22	,,
6	2.4	3.6	1.8	7.8	,,
7	18	15	3.7	—	Haematemesis, jaundice
8	7.8	6.8	3.8	150	,,
9	5.6	3.0	3.4	12	Hepatitis
10	2.5	2.7	2.5	—	,,
11	4.5	2.5	4.0	—	,,
12	4.8	4.4	3.0	28	,,
13	4.5	6.7	2.3	20	,,
14	12	11	1.4	21	,,
15	3.0	6.0	1.1	21	,,
16	22	15	5.6	19	Hepatomegaly
17	13	15	4.8	82	Obstructive jaundice
18	8.8	6.2	5.6	63	,, ,,
19	8.2	4.5	3.0	32	,, ,,
20	7.5	3.8	3.8	21	,, ,,
21	7.2	6.5	5.7	136	,, ,,
22	6.3	6.3	4.9	37	,, ,,
23	6.0	5.3	4.5	17	,, ,,
24	6.0	3.6	1.8	40	,, ,,
25	5.4	3.6	3.0	23	,, ,,
26	5.2	3.8	4.5	31	,, ,,
27	3.8	3.8	2.5	14	,, ,,
28	3.3	2.3	1.8	21	,, ,,
29	2.7	1.8	1.8	80	,, ,,
30	2.6	1.2	0.7	20	,, ,,
31	19	14	7.5	44	,, ,,
32	6.3	3.8	1.8	90	,, ,,
33	6.0	3.5	4.5	40	,, ,,
34	2.7	1.8	1.8	80	,, ,,
35	2.6	1.3	1.9	40	Ulcerative colitis
36	8.0	5.3	6.0	114	Xanthomatosis
37	7.6	5.6	4.9	105	,,
38	3.2	3.2	3.0	122	,,

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/100 ml.)	Notes
	Total	Alcohol-stable	Formaldehyde-stable		
MISCELLANEOUS DISEASES					
1	6.0	—	3.0	—	Anaemia
2	1.5	1.5	1.5	—	"
3	6.0	4.5	3.0	—	Anaemia haemolytic
4	3.8	—	1.5	17	" "
5	3.6	7.8	2.4	6	" "
6	16	—	5.2	—	" and oedema
7	1.2	1.2	1.2	19	" pernicious
8	1.8	1.8	1.8	—	" "
9	5.0	3.8	1.4	—	" splenic
10	1.8	2.6	0.3	—	Arthritis
11	1.8	1.2	0.7	—	"
12	1.6	—	0	—	" (rheumatic)
13	24	19	1.8	17	Bronchopneumonia
14	8.7	—	1.5	—	"
15	6.2	—	1.8	—	Cystitis "
16	2.3	2.3	1.4	11	Diabetes
17	2.0	0.5	0.5	—	" and uraemia
18	3.5	2.8	2.8	—	Dysurea
19	3.8	—	2.3	—	Epididymitis
20	4.8	4.2	2.0	5.5	Fracture
21	3.7	3.7	2.2	—	Gout
22	8.7	7.5	1.5	—	Heart failure
23	4.2	—	0	10	" "
24	2.2	2.0	0.7	—	" "
25	2.5	—	1.6	—	" " and hypertension
26	12	—	6.0	—	Haemorrhage
27	2.2	1.5	1.8	—	" accidental
28	2.3	1.5	1.3	—	" "
29	17	19	4.5	—	Haematemesis
30	7.5	6.8	3.0	—	"
31	4.7	3.3	2.7	—	Haematuria
32	2.4	1.8	0	34	Hodgkin's disease
33	5.9	8.5	3.1	12	Hypertension
34	2.2	—	0.7	20	Hypertensive heart disease
35	7.0	7.0	3.0	—	Hydronephrosis

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/100 ml.)	Notes
	Total	Alcohol-stable	Formaldehyde-stable		
MISCELLANEOUS DISEASES (*continued*)					
36	4.8	4.2	3.0	17	Melaena
37	1.8	1.3	0	6.6	"
38	25	17	0	14	Myeloid leukaemia
39	4.5	4.5	2.2	—	Myxoedema
40	1.5	—	0.4	—	"
41	12	8.0	6.0	—	Nephritis
42	11	11	3.7	—	"
43	2.5	—	0.8	—	"
44	2.3	9.7	1.5	—	"
45	2.2	2.1	0.8	—	"
46	1.8	1.8	0.7	—	"
47	1.7	1.7	1.7	—	"
48	8.7	6.7	5.3	—	" chronic
49	5.3	3.8	1.5	—	" "
50	2.0	1.5	1.5	—	" "
51	1.8	4.2	1.8	9.6	" "
52	3.5	3.5	2.0	—	Nephrosis
53	6.0	6.7	1.8	—	Toxaemia of pregnancy
54	3.3	1.2	1.2	—	" " "
55	1.5	1.5	1.5	—	" " "
56	1.2	1.2	0.3	—	" " "
57	3.3	—	1.9	—	Papilloma of bladder
58	3.0	4.5	0	18	Peritonitis
59	16	—	5.2	—	Pyloric ulcer
60	12	—	6.6	48	Steatorrhoea
61	3.8	3.5	1.5	60	"
62	1.5	—	1.5	—	Stenosis, mitral
63	15	9.8	3.4	—	Stricture, urethral
64	2.6	1.8	1.8	—	Sprue
65	3.7	1.5	0.8	—	Hypertension
66	1.7	2.2	0.4	—	Thyrotoxicosis
67	1.3	—	0.4	—	Ulcerative colitis
68	3.8	3.8	1.1	10	Undiagnosed,
69	2.7	6.0	2.0	—	"
70	4.5	—	0	—	"
71	2.7	—	0.7	11	"
72	6.0	3.0	1.5	—	Uraemia
73	2.1	1.4	1.0	17	Wilson's disease

J. clin. Path. (1948), **1**, 91.

A QUANTITATIVE PRECIPITATION TEST FOR SYPHILIS

BY

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(RECEIVED FOR PUBLICATION, MARCH 26, 1947)

In February, 1946, under the title of "A Preliminary Report on a Modification of the Kahn Test" (Price, 1946a), the author described a modification of the Kahn test, the advantages of which were stated to be: (1) that the results are more easily read than those of the Kahn test; (2) the results given by antigens prepared at different times and having different titres are identical; (3) the results obtained by this test appear to be somewhat more specific than those given by either the Kahn or Wassermann reactions. After more than 3,000 tests there seems to be no reason to modify these opinions.

One major disadvantage remained, that is, the difficulty in the preparation of the alcoholic extract of dried heart muscle according to Kahn's technique. If, moreover, one considers the production of the dried heart powder itself, the manufacture of Kahn antigen is outside the scope of most pathological laboratories. It is, therefore, desirable from all points of view to attempt to prepare an antigen which can be made in any laboratory, is specific in action, reasonably sensitive, and easy to handle.

Experimental

The starting point was the standard cholesterolized alcoholic extract of heart muscle used in the Harrison–Wyler (1929) technique of the Wassermann reaction. The lipoids were precipitated as described in the previous paper, and an attempt was made to work out a precipitation titre (Price, 1946b). Unfortunately, whilst positive sera gave fairly strong reactions all the negative serum and saline tubes contained a faint precipitate which was thought to be due to excess of cholesterol.

Accordingly, in the next experiment, Wassermann reaction alcoholic heart extracts containing varying quantities of cholesterol from 0.05 to 0.3 per cent were used. The precipitation titres obtained with the various antigens were:

W.R. alcoholic extract +	0.05% cholesterol	1 to 0.8 saline
,, ,, ,,	0.1% ,,	1 to 1.0 saline
,, ,, ,,	0.2% ,,	1 to 1.4 saline
,, ,, ,,	0.3%* ,,	no titre obtainable; fine precipitate in all tubes.

*It should be pointed out that cholesterol at 0.3 per cent strength in the alcoholic extract required heat at 37° C. to effect solution and on regaining room temperature the cholesterol precipitated out from the solution.

When these antigens were tested by the method of optimal proportions (Price, 1946b), the best results were obtained when the ratio of serum to antigen was 5 to 1 and the precipitate of lipoids from 1 ml. of the heart extract (cholesterolized) obtained by using the precipitation titre was suspended in 0.6 ml. of saline. On the other hand, when used in a test, the less the alcoholic extract was cholesterolized the larger became the precipitates obtained with positive sera. Thus the best reaction was obtained with the alcoholic extract of heart muscle containing 0.05 per cent cholesterol.

Plain uncholesterolized alcoholic extract of ox heart was then used as an antigen in the manner already described for the cholesterolized extracts. The results of these experiments are summarized in Table I.

TABLE I

Alcoholic extract of ox heart 9 ml. to the gramme	Precipitation titre	Serum titre of reacting serum
Plain	0.8 ml. saline to 1 ml. heart extract	80 units
Plain + 0.05% cholesterol	0.8 ml. saline	80 units
Plain + 0.1% cholesterol	1.0 ml. saline	40 units
Plain + 0.2% cholesterol	1.4 ml. saline	20 units

It is evident that cholesterol as a sensitizer of this type of antigen was unimportant and could be omitted. The Wassermann reaction alcoholic extract of heart muscle used in the above experiments was obtained by extracting fresh wet ox heart muscle, cleaned from fat, with 9 ml. of absolute alcohol per gramme of muscle for 24 hours at 37° C. After filtration the alcoholic extract was placed in the ice chest (4° C.) for twenty-four hours and then refiltered.

Variations in the method of extraction were now investigated. Wet ox heart muscle was extracted for twenty-four hours, three days, and five days at 37° C. and at room temperature for three and five days respectively. All these extracts gave identical precipitation titres, and if cholesterol were added in the various amounts mentioned above (0.05 per cent, 0.1 per cent, 0.2 per cent) the precipitation titres varied accordingly. Furthermore, if serum quantitative tests on positive sera

were carried out using the same serum for each antigen, the unit values obtained varied in a similar manner to those obtained with the original extracts. In short, the method of extraction did not affect the precipitation reaction.

During these experiments it was noticed that when heat was employed in the extraction a deposit of crystals separated out from the alcoholic extract after about six weeks. These crystals could not be removed by filtration or centrifugalization, and they interfered with the reading of the tests as it was found that all tubes contained a fine deposit when examined with a $\times 6$ lens. Up to the time that crystals started to appear there was no interference with the test and they were regarded as a non-specific by-product of the heat-induced alcoholic extraction. As a result of these experiments the use of heat in the alcoholic extraction of the heart muscle was abandoned and extraction at room temperature was adopted as the routine method.

Hitherto all extracts had been prepared by extracting one gramme of the ox heart muscle with 9 ml. of absolute alcohol. The next step was to ascertain what effect, if any, varying amounts of absolute alcohol per gramme of wet ox heart muscle would have on the quality of the antigen. The amounts used were 2.5 ml., 5 ml., and 15 ml. per gramme respectively. The results are shown in Table II.

The titrations given in the table below were repeated several times and the results obtained were always identical for the precipitation titres. At the same time unit values of sera of different strengths always bore the same proportion.

From these experiments it was concluded that

1. An alcoholic extract of ox heart obtained by using 2.5 ml. of absolute alcohol per gramme of muscle is useless as an antigen in this test.
2. The best results are obtained when an extract obtained by using 5 ml. of absolute alcohol per gramme of ox heart muscle is used.
3. The addition of cholesterol to the alcoholic extract of heart muscle in amounts of 0.05 per cent had no sensitizing action. Above this amount it inhibits the sensitivity of the extract as an antigen.
4. Whatever amount of absolute alcohol per gramme of ox heart is used to prepare an antigen, the best results are always obtained by using the plain extract without the addition of cholesterol.

It now remained to be shown which extract is the most sensitive antigen. Since plain alcoholic extracts

TABLE II

Each gramme of wet ox heart muscle extracted by	Antigen	Precipitation titre in terms of normal saline to each ml. of heart extract	Value of given serum (in units)
2.5 ml. of absolute alcohol ..	Plain extract	None obtainable: precipitate in all tubes	—
	Plain extract + 0.05% cholesterol	,, ,,	—
	Plain extract + 0.1% cholesterol	,, ,,	—
	Plain extract + 0.2% cholesterol	,, ,,	—
5 ml. of absolute alcohol	Plain extract	0.6 ml.	320
	Plain extract + 0.05 cholesterol	0.6 ml.	320
	Plain extract + 0.1% cholesterol	0.8 ml.	80
	Plain extract + 0.2% cholesterol	1.0 ml.	40
15 ml. of absolute alcohol ..	Plain extract	0.8 ml.	20*
	Plain extract + 0.05% cholesterol	1.0 ml.	20
	Plain extract + 0.1% cholesterol	1.2 ml.	20
	Plain extract + 0.2% cholesterol	1.6 ml.	20

*Whilst this titre could not be read at higher than 20 units, the precipitate was somewhat heavier than those given by the other antigens in the same class.

of ox heart muscle had always proved the most sensitive of their group (with or without cholesterol added), the three plain extracts obtained by using 5 ml., 9 ml., and 15 ml. of absolute alcohol respectively per gramme of heart muscle were used as antigens in parallel with different sera of varying strengths. Many hundreds of sera were tested, but Table III gives a sample of ten sera tested at the same time.

The ten sera shown in the table were unselected except in so far as they came from one batch of sera tested by the Wassermann reaction. Of the antigens employed, it will be seen that that obtained by extracting 1 g. of ox heart muscle with 5 ml. of absolute alcohol is the most sensitive, whilst between the other two there is little to choose. These results were repeated many times with similar results.

To conclude the experimental work the sensitivity of the antigen as described in the first paper was compared (Price, 1946c) with the sensitivity of the antigen prepared in the above manner. More than 1,000 sera were tested in parallel, and it was found that sera yielding 0 units with the one antigen always gave a similar result with the other. On the other hand, when sera yielding positive results were encountered, considerably higher values were given by the latter antigen than by the former. This may perhaps be best shown in the form of a graph (Fig. 1).

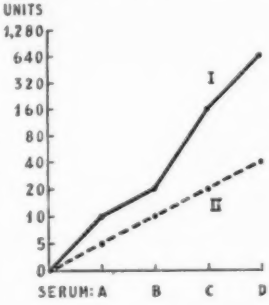

FIG. 1.—(I) Plain alcoholic heart extract antigen used. (II) Antigen prepared from Kahn extract.

The Test Proper

The above experimental work is a continuation of that contained in the first paper (Price, 1946a), and the test about to be described, henceforth referred to as the P.P.R.* for syphilis, is based on the technique as set out therein.

Apparatus required.—The necessary apparatus is as follows: Wassermann tubes 5 cm. × 1.25 cm.; Wassermann racks to hold tubes; pipette† to hold 5 volumes (0.11 ml.); droppers† to hold 5 volumes (0.11 ml.), for saline used in conjunction with Donald's dropping apparatus; 1 volume (0.022 ml.); shaking apparatus, 280 oscillations per minute.

*Price's Precipitation Reaction.

†The above volume (0.022 ml.) is purely arbitrary and was adopted since the necessary pipette and droppers were already employed in Harrison Wyler technique of the Wassermann reaction (M.R.C. report 1929) used in this laboratory. In addition it ensured a reasonable economy of serum and antigen.

TABLE III

Serum No.	Antigen prepared by extracting 1 g. of ox heart with 5 ml. of absolute alcohol	Antigen prepared by extracting 1 g. of ox heart with 9 ml. of absolute alcohol	Antigen prepared by extracting 1 g. of ox heart muscle with 15 ml. absolute alcohol	W.R. result	Kahn test result
1	40 units	10 units	10 units	++	Positive
2	80 units	10 units	10 units	++	Positive
3	320 units	320 units	320 units	++	Positive
4	40 units	5 units	5 units	++	Positive
5	0 units	0 units	0 units	Neg.	Negative
6	10 units	5 units	5 units	++	Positive
7	320 units	160 units	80 units	++	Positive
8	320 units	40 units	40 units	++	Positive
9	160 units	40 units	40 units	++	Positive
10	40 units	5 units	5 units	++	Positive

Preparation of the alcoholic heart extract.—An ox heart obtained from a freshly killed beast is obtained, stripped of fat, cut into small pieces, and weighed. The muscle is ground in a mortar for one minute with a few ml. of the alcohol and glass powder. The mixture is then transferred to a suitable glass-stoppered bottle and the mortar washed out with a further few ml. of the alcohol, the washings being then poured into the bottle. Sufficient alcohol is now added so that the bottle contains 5 ml. of alcohol for every gramme of heart muscle. The bottle is well shaken for a few seconds and placed in a dark cupboard or box for three days at room temperature, being shaken two or three times each day. At the end of this time the contents of the bottle are filtered into another glass-stoppered bottle, which is then placed in the ice chest (4° C.) for 24 hours. Finally, whilst still cold, it is refiltered into a bottle of brown-coloured glass (to exclude light). The very pale-lemon-coloured filtrate is then ready for use.

Precipitation titre.—Before the antigen can be prepared for use in the test proper, the precipitation titre must be determined. This is done as follows. Four mixtures of 1 ml. of the alcoholic heart extract are made with respectively 0.4 ml., 0.6 ml., 0.8 ml., and 1 ml. of normal saline. The mixtures should be made by gently pouring the alcoholic extract down the inside of the small tube containing the saline and then pouring back into the tube which originally contained the extract. Gentle but thorough mixing is obtained by pouring and repouring seven times in all. The mixtures are allowed to stand for 30 minutes. The precipitate which forms in each tube is packed tightly into the bottom of the tube by centrifugalization (2,000 r.p.m.) for ten minutes, at the end of which time the supernatant fluid can be removed without fear of loss of any of the precipitate. The tubes are allowed to drain for five minutes by standing in the inverted position. Normal saline (0.6 ml.) is added to each precipitate, and thorough mixture obtained by means of a Wright's capillary pipette. Thus four antigens are obtained, and these are titrated by adding one volume of each antigen to five volumes of known moderately positive serum, one volume to five volumes of normal serum, and one volume to five volumes of saline. After five minutes on a shaking machine, 1 ml. of saline is added to each tube. The rack containing the tubes is gently agitated and the results read by means of a ×6 lens and a slit lamp. The precipitation titre is indicated by the tubes showing no precipitate and containing the antigen obtained by adding the least amount of saline to 1 ml. of the alcoholic heart extract. These tubes containing no precipitate are of course always in the normal serum and saline rows. The tubes containing moderately positive serum are not essential for the titration but are included for the sake of completeness.

This titration may be summarized as in Table IV. From the Table it is clear that the least amount of saline to be added to 1 ml. of the alcoholic extract (5 ml. to the gramme) of ox heart muscle in order to obtain a suitable antigen is 0.6 ml., and this constitutes the precipitation titre. It is a matter of experience that, although very many of these extracts have been titrated, the precipitation titre has always been 0.6 ml. of normal saline to 1 ml. of the alcoholic extract of ox heart muscle. Furthermore, once this titre is determined for any given extract it remains constant. The amount of normal saline (0.6 ml.) added to the precipitate to form the antigen has already been determined by the method of optimal proportions (Price, 1946b).

Preparation of antigen for use in the test proper.—Having ascertained the precipitation titre as 0.6 ml. of normal saline to 1 ml. of heart extract, a simple proportion sum will give the amounts of each reagent to mix to produce the amount of antigen necessary for a given number of tests. The practice in this laboratory is to make up enough for at least a week's work. To make 12 ml. of the antigen (enough for 300 to 400 serum tests) add 20 ml. of the alcoholic heart extract to 12 ml. of normal saline. This operation is performed by measuring 20 ml. of the alcoholic extract in one 50 ml. centrifuge tube and 12 ml. of normal saline into a similar tube. The alcoholic extract is first poured on to the saline, and then the mixture is poured back and forth six times, subsequently being allowed to stand for 30 minutes. (It is of the utmost importance that the *alcoholic extract* be *poured on to the saline* in the first instance, otherwise a very fine non-specific precipitate is apt to appear in all tubes of the test. This may confuse the reading.

TABLE IV

Alcoholic extract of ox heart 5 ml. to 1 g.	Normal saline added to obtain precipitate	Saline added to precipitate to form antigen	Reaction precipitate		
			Syphilitic serum	Normal serum	Saline
1 ml.	0.4 ml.	0.6 ml.	+	+	±
1 ml.	0.6 ml.	0.6 ml.	++	Nil	Nil
1 ml.	0.8 ml.	0.6 ml.	++	Nil	Nil
1 ml.	1.0 ml.	0.6 ml.	+±	Nil	Nil

especially at the end-points of quantitative tests.) At the end of this time the precipitate which has formed is swung to the bottom of the tube by centrifugalization (2,000 r.p.m.) for 10 minutes. The supernatant fluid can then be poured off with safety and any remaining fluid allowed to drain out by placing the tube for five minutes on a filter paper in the inverted position. Any fluid still left on the sides of the tube can be wiped off with a towel and 1.5 ml. of normal saline added to the precipitate at the bottom of the tube. The mixing of the precipitate and the saline is accomplished by means of a Wright's capillary pipette, and the resulting suspension is transferred to a brown glass bottle either with a glass stopper or vulcanite screw cap. Tinfoil stoppers are to be avoided, since the antigen is acid enough to set up a reaction with the metal. In order to avoid infection of the made-up antigen, 0.022 ml. of sodium azide, 6.8 per cent, is added to each ml. of the suspension. When not in use the antigen should be kept in the ice chest at 4° C. In addition the antigen pipette should have a cotton-wool plug at the teat end and should be rinsed out with acetone and dried before use. If kept thus, the antigen remains active for months.

Sera.—The sera to be tested are obtained in exactly the same manner as those subjected to a Wassermann reaction and are inactivated for thirty minutes at 56° C.

Screen Test

Each serum to be tested has two tubes allotted to it. Into one is put 5 volumes of neat serum and into the other 5 volumes of serum diluted 1 in 2 with saline. When testing large numbers of sera it is advisable to use the saline dropper in conjunction with Donald's dropping apparatus. This will deliver automatically five volumes of saline per drop, and this five volumes of saline can be rapidly delivered into each tube of the back row. Five volumes of the appropriate neat serum can then be added to each of the respective tubes of the front and back rows, five volumes from the latter being discarded after thorough mixing.* One volume of antigen is now added to all tubes necessary for the batch of sera under test.

Screen Test

Tube 2	5 volumes of serum diluted 1 in 2 1 volume of antigen
Tube 1	5 volumes of neat serum. 1 volume of antigen.

The rack containing the tubes is placed for five minutes in a shaking apparatus (280 oscillations per minute). Subsequently 1 ml. of normal saline is added to each tube. The contents of the tubes are now well mixed by gentle agitation of the rack, and the results read by means of ×6 lens and the slit lamp. When reading results the tube should be held on the slant and the particles, if any, looked for in the elliptical disc formed by the top of the fluid. The lowest positive reading is recorded if small discrete greyish particles of similar size are seen. Stronger reactions cause a greater number of particles which agglutinate and form a greyish-white precipitate, whilst the strongest reactions result in particles easily visible to the naked eye. These particles appear as large white flocculi. In tubes in which no reaction takes place the fluid contains no particles and is of a uniform clarity.

The sera giving negative results are recorded, and those giving positive results are dealt with by means of the quantitative test.

Quantitative Test

The quantitative test is set up on the principle of diluting the serum by a series of twofold dilutions.

Four tubes are allotted to sera giving weak reactions, and eight tubes to those giving strong reactions. All tubes except the first receive five volumes of saline, and if large numbers of positive sera are to be dealt with it is advisable to use the saline dropper in conjunction with Donald's dropping apparatus. The first and second tubes each receive five volumes of the serum to be tested. The contents of tube 2 are well mixed, and five volumes are carried over to tube 3. The mixing and carrying is repeated from tube to tube in serial order until the last one is reached, when five volumes of the mixture are discarded leaving five volumes of this dilution in the tube. Thus the series of tubes allotted to each serum contains one of neat serum, and dilutions from 1 in 2 to 1 in 128 depending on the number of tubes used. All tubes now receive one volume of the antigen and are shaken for five minutes on the shaker.

Strongly Reacting Serum		*Quantitative Test*	
5 volumes of serum diluted			
Tube 4	1–8	Tube 5	1–16
Tube 3	1–4	Tube 6	1–32
Tube 2	1–2	Tube 7	1–64
Tube 1	Neat serum	Tube 8	1–128

*It had been hoped to make the screen reaction a one-tube test using neat serum alone. With experience it was found that some strongly reacting sera contained too much reagin for the amount of antigen employed, thus suppressing the precipitation reaction. This zone phenomenon can be overcome successfully by using the two-tube technique.

With high titre serums the dilutions may have to be carried further.

One ml. of saline is then added to each tube by means of the simple apparatus shown in Fig. 2.

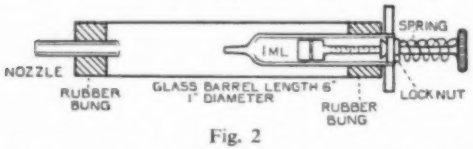

Fig. 2

The barrel of the apparatus holds approximately 30 ml. of saline, and each time the plunger is pushed home against the spring 1 ml. is delivered through the nozzle when once the locknut on the plunger inside the small syringe has been suitably adjusted. This simple apparatus saves much time and labour. Mixing of the contents is ensured by gentle agitation before reading with $\times 6$ lens and slit lamp. The tube containing the highest dilution of the serum which gives the lowest positive reading (that is, small discrete greyish particles of similar size seen in the elliptical disc at the top of the slanted tube) is noted. From this the number of units is calculated. For example, if the tube containing neat serum is the only one to show any particles (this is the lowest positive reading) the titration is recorded as 5 units on the grounds that no serum test is proven to be sensitive enough to demonstrate the extreme limits of the small amounts of reagin present in some syphilitic sera. If, on the other hand, the highest dilution of serum recording a positive reaction is 1 in 64, then the titre of the serum is recorded as 320 units. Occasionally sera from patients suffering from florid secondary syphilis or multiple gummata record positive readings above the eight-tube dilution, the record so far being the tenth tube (2,560 units).

Thus the readings of the test are recorded in terms of units such as 0, 5, or multiples of five, and are designed to give a rough idea of the relative activity of the disease.

Cerebrospinal Fluid

This test has been adapted to testing the cerebrospinal fluid, and, although the numbers tested as yet are relatively small, no fluid has been met which recorded any degree of positivity with the Wassermann reaction and failed to record a positive P.P.R. for syphilis.

The technique is similar to that used with the serum, and is as follows:

. By the principle of optimum proportions it has been ascertained that the best results are obtained when 15 volumes of cerebrospinal fluid are used with 1 of antigen.

Screen test.—Each cerebrospinal fluid has one tube allotted to it, into which one volume of antigen and 15 volumes of cerebrospinal fluid are placed and shaken for five minutes. At the end of this time 0.5 ml. of saline is added to each tube, the contents of which are mixed by gentle agitation and the results read in the manner described for sera. All positively reacting fluids are put up for a quantitative test.

Quantitative test.—Each cerebrospinal fluid is allotted eight tubes. All tubes excepting the first receive fifteen volumes of saline, and the first and second tubes receive fifteen volumes of cerebrospinal fluid each. Serial dilutions of from 1–2 to 1–128 are made by mixing and carrying over fifteen volumes from tube to tube until the last is reached, when, after mixing, fifteen volumes of the mixture are discarded. Thus each positive cerebrospinal fluid has a series of tubes containing fifteen volumes of the neat fluid and fifteen volumes of a series of dilutions from 1–2 to 1–128. Each tube now receives one volume of the antigen and is shaken for five minutes, and subsequently 0.5 ml. of saline is added. Mixing of the contents of the tube is secured by gentle agitation and results read and recorded in the same manner as for sera.

Cerebrospinal fluids require no treatment and are suitable for test provided they are reasonably free from foreign matter. The highest recording from a cerebrospinal fluid to date is 160 units.

Results

Over 8,000 routine tests have been done in parallel with the Wassermann reaction and Kahn test, with extremely satisfactory results. In order to assess the value of this test as against the Wassermann and Kahn tests, the results of 2,936 routine parallel tests have been examined. These tests were the yield of three months' routine testing and are unselected. The results were as follows:

Agreements

Negative reactions	..	2,459 (83.7%)
Positive reactions	..	358 (12.2%)
Total	..	2,817 (95.9%)

Disagreements 119 (4.1%)

Of these the following analysis may be made:

Group 1.	P.P.R.	negative	
	Wassermann reaction	negative	
	Kahn reaction	positive	66 sera.

Group 2.	P.P.R.	positive	
	Wassermann reaction	negative	
	Kahn reaction	positive	50 sera.
Group 3.	P.P.R.	positive	
	Wassermann reaction	negative	
	Kahn reaction	negative	1 serum.
Group 4.	P.P.R.	negative	
	Wassermann reaction	positive	
	Kahn reaction	negative	1 serum.
Group 5.	P.P.R.	positive	
	Wassermann reaction	positive	
	Kahn reaction	negative	1 serum.

Examination of the case cards of those patients whose sera gave conflicting results reveals that they were grouped into the following six types of case:

1. *Primary syphilis untreated.*—In this group there were 3 sera, all of which recorded positive Kahn tests. One serum reacted positively with the P.P.R. and negatively with the Wassermann reaction, whilst two sera gave negative results with these two tests.

2. *Latent syphilis treated.*—This was the largest class, consisting of 95 sera. All the patients had been treated with penicillin followed by arsenic and bismuth. All the sera recorded negative Wassermann reactions. There were 3 results which were possible false positive Kahn reactions. For the rest, the noticeable feature was that usually the Wassermann became negative first, then the P.P.R., which was followed a month or two later by the Kahn test. A reversal of a negative P.P.R. to positive occurred on only two occasions.

3. *Latent syphilis untreated.*—In this group there were only two sera, both of which recorded negative Wassermann reactions, but positive P.P.R.s and Kahn tests.

4. *Old cured syphilis.*—One serum was tested from a patient who had had syphilis in 1924. He came up for a check blood test, but showed no signs or symptoms. In a series of three tests done within a month the Kahn was positive on one occasion, whilst the Wassermann and P.P.R. were negative at each testing.

5. *Patients attending for treatment for gonorrhoea.*—There were 12 of these sera, 9 of which recorded negative Wassermann and P.P.R. reactions, but positive Kahns. Of these 9 patients, who were under observation for months, none revealed any clinical or serological evidence of syphilis, apart from the Kahn test. The remaining 3 were problem sera. One gave persistently positive Kahn and P.P.R. results with negative Wassermann reactions over a period of three months. He was given 150,000 units of penicillin at the outset of his gonorrhoeal infection, but after two months there was no evidence of syphilis. He remains under observation. Patient 2 was a coloured patient who reported that he had had yaws as a youth. He attended with gonorrhoea and received 150,000 units of penicillin, followed by a further 200,000 units. Over a period of three months he persistently recorded negative Wassermann and positive Kahn and P.P.R. reactions. There was no clinical evidence of syphilis, and he remains under observation. Patient 3 attended with gonorrhoea, recording a negative Wassermann but a positive Kahn and P.P.R. There was no evidence of syphilis and he was given 150,000 units of penicillin. Six days later his blood recorded all three tests as positive. Unfortunately the patient defaulted so no opinion could be given, but possibly he had a double infection of gonorrhoea and syphilis, the latter being first detected by the P.P.R. and Kahn tests.

6. *Sera from patients in whom no evidence of venereal disease was found.*—There were six of these sera, all of which recorded negative P.P.R. and Wassermann reactions but positive Kahn tests. None of these patients whilst under observation revealed any evidence of syphilis in spite of repeated clinical examination and blood tests.

Primary Syphilis

In order to ascertain how soon after infection the P.P.R. becomes positive, the case records of all patients attending the Whitechapel Clinic and suffering from untreated primary syphilis during a period of six months were examined. The number totalled 80. *Spirochaeta pallida* had been demonstrated in the primary lesions in all cases.

Although it is difficult to be certain of the exact date of the infecting exposure, it was thought to be of sufficient interest to attempt to ascertain how soon after an exposure a positive P.P.R. would be recorded. Of 38 patients in the first four weeks after exposure, the serum of 19 (50 per cent) recorded a positive P.P.R. Of 29 patients in the fifth to eighth week after exposure the sera of 16 (55 per cent) recorded positive P.P.R. reactions. Finally, of 13 patients in the ninth to twelfth weeks after exposure the sera of 7 (53 per cent) recorded positive P.P.R. reactions. It should be noted that the parallel Wassermann reactions and Kahn tests were identical.

Cerebrospinal Fluid

During this investigation a total of 284 cerebrospinal fluids were tested in parallel with the Wassermann reaction. Of these, both tests recorded 13

positive and 271 negative reactions. The highest unit value yet recorded by the test with cerebrospinal fluid was 160, and it appears that values of 5 to 10 units suggest a condition of tabes dorsalis, whilst higher values are more typical of fluids obtained from patients suffering from general paralysis of the insane.

Comment

Whilst this examination of results is not regarded as exhaustive, it does seem to show that the P.P.R. is at least as sensitive as the Wassermann reaction and somewhat more specific than the Kahn test. Thus, of 9 patients suffering from gonorrhoea and 6 in whom no signs of venereal disease were discovered, all recorded negative P.P.R. but positive Kahn tests. These latter seem to be false positives, particularly as the Wassermann as well as the P.P.R. reactions were negative. They represent 15 (12 per cent) out of the total of 119 conflicting results recorded by parallel Wassermann, Kahn, and P.P.R. reactions respectively, or 0.5 per cent of the total number of sera (2,936) examined. Of these same sera only 2 appeared to give false positive P.P.R. reactions.

Consideration of the results obtained by this test suggests that the quantitative test gives some indication of the activity of the disease. In general terms it may be said that the greater the number of units recorded by a serum, the more active is the spirochaetal infection. Sera from patients suffering from florid secondary syphilis or multiple gummata always record a high unit value, and the cerebrospinal fluids from patients suffering from general paralysis of the insane record higher readings than the cerebrospinal fluids of tabetics.

Summary

1. The experimental work underlying a serum precipitation test for syphilis is given.

2. The preparation of the alcoholic extract from the raw ox heart is described. This operation is well within the capacity of any moderately well-equipped laboratory.

3. The preparation of the antigen from the alcoholic extract of ox heart and its standardization for use in the test is given in detail.

4. The screen test as described is simple, speedy, reliable, and easily read.

5. The method for the quantitative test is given; the test gives some idea of the activity of the disease.

6. The testing of cerebrospinal fluids (which require no prior preparation) is shown to give results equal to those of the Wassermann reaction.

7. The results of 2,936 routine parallel tests with the Wassermann and Kahn tests are examined.

I would like to express my indebtedness to Mr. Tweed, senior technician to the V.D. Reference Laboratory, and to Dr. Wilkinson, assistant pathologist to the Whitechapel Clinic, for much help in the routine work of this investigation.

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CHLORAL HYDRATE PLATES FOR THE INHIBITION OF SWARMING OF PROTEUS

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(RECEIVED FOR PUBLICATION, JULY 17, 1947)

In routine bacteriology the swarming of *Proteus* on plate cultures often masks the identity or presence of other bacteria which may be present. Mixed cultures including *Proteus* are often obtained from open wounds or sinuses following abdominal or chest operations, so that unless some special selective medium is used it is usually impossible to identify or to isolate other bacteria from a culture containing *Proteus*. Frequently β-haemolytic streptococci are missed in such cultures, and thus one often fails to identify an organism causing some infection which may have become secondarily infected with *Proteus*.

Numerous methods have been published for the inhibition of swarming of *Proteus* on culture plates, the simplest being the use of 6 per cent agar (Hayward and Miles, 1943). This high concentration of agar often leads to technical difficulties in preparation, and if the plates are not sufficiently dried, or if a very moist specimen is inoculated, *Proteus* is not necessarily prevented from swarming. Many chemicals have been incorporated in agar to make selective media, the most popular method being that of adding 1/5,000 sodium azide (Snyder and Lichstein, 1940). Unfortunately, when sodium azide blood-agar plates are used the blood tends to haemolyse and the plates do not keep satisfactorily before use. Jones and Handley (1945) described a satisfactory highly selective medium for the isolation of *Salmonellae* from material contaminated with *Proteus*.

In 1931 Krämer and Koch reported the results of their experiments using 14 different types of media for the inhibition of swarming, and showed how the addition of chloral hydrate prevented the swarming of *Proteus*. In this country the practical use of chloral hydrate plates appears to be little known except in certain laboratories; this paper has therefore been written in order to bring it to the knowledge of everyone as being a practical method.

After certain preliminary experiments it has been found that chloral hydrate in a final concentration of 1/1,000 in nutrient agar or blood agar inhibits the swarming of *Proteus* but allows good growth of *Proteus* and of most other bacteria. Experience with the method coincides with that of Krämer and Koch, who found that practically all bacteria produced typical colonies. One great advantage is that it allows good growth of β-haemolytic streptococci, and also permits the production of β haemolysis, which thus enables one to detect and isolate single colonies from cultures heavily infected with *Proteus*. It is an advantage to incubate the plates for a full 24 hours, although after 18 hours the β haemolysis of haemolytic streptococci can usually be detected with ease.

On 1/1,000 chloral hydrate plates colonies of *Proteus* appear as discrete colonies from 1 to 2 mm. in diameter, so that if necessary one can pick off single colonies of *Proteus* and also single pure colonies of other bacteria from mixed cultures. This is an advantage over media which inhibit the growth of *Proteus*, as on them colonies of other bacteria need not necessarily be pure, as the *Proteus* bacilli just fail to grow but lie dormant and live on the plate. Weaker concentrations, such as 1/1,500, allow the colonies of *Proteus* to become large and semi-confluent, making it difficult to isolate other types of colonies present. At 1/2,000 slight spreading occurs.

Stronger concentrations inhibit the growths of *Proteus* and of other bacteria, but also inhibit the production of β haemolysis on blood-agar plates by haemolytic streptococci.

Preparation of Plates

The method of preparation of chloral hydrate plates is simple. A stock 10 per cent solution of chloral hydrate is made in distilled water and an appropriate amount added to the medium to give a final strength of 1/1,000. It is an advantage to make several plates at one time, adding the chloral hydrate to the bulk base at the time of pouring the plates, otherwise a very small volume must be added to the medium for each plate. It is not necessary to sterilize the stock solution, as it is apparently self-sterilizing at this strength, but it is an obvious advantage to exclude bacteria, especially spore-bearers, by using sterile distilled water. If preferred, the stock solution may be autoclaved without interfering with its inhibitory properties. Once the plates are poured they should be dried in the usual way, but not overdried.

The chloral hydrate blood-agar plates may be stored in the refrigerator for a week before use without the occurrence of haemolysis. Any stored plate should be dried in the incubator for about half an hour before inoculation.

Krämer and Koch report having found occasional batches of blood-agar plates giving atypical growths, but over the last three years no very unusual growth has been found as long as the correct percentage is accurately added to the medium. Slight increase in the concentration due to the inaccurate addition of too small a volume of stock solution will lead to irregular results.

Conclusions

1. Chloral hydrate in a final concentration of 1/1,000 in nutrient agar or blood-agar plates inhibits the swarming of *Proteus*.

2. The chloral hydrate in the strength recommended permits the growth of *Proteus* as single colonies 1 to 2 mm. in diameter.

3. Chloral hydrate in this strength allows the production of β haemolysis on blood-agar plates by haemolytic streptococci.

4. Chloral hydrate plates permit the identification and isolation in pure culture of bacteria from mixed cultures contaminated with *Proteus*.

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REVIEWS

Illuminants and Illumination for Microscopical Work. Monograph III of the Quekett Microscopical Club. By F. E. Ockenden. London. Williams and Norgate: for the Quekett Microscopical Club, 1947. Pp. 26. Price 2s. 6d. net.

Most microscopists know little of the principles which determine the choice of illuminants and illumination for microscopical work. The third of the Quekett Microscopical Club's monographs should, therefore, be of considerable interest to all those who use the microscope and are anxious to obtain the best critical illumination possible.

Before light sources are discussed, various terms and definitions are given, such as "Candle" (symbol I), "the standard of luminous intensity," "Flux" (symbol F) and "foot candle" (f.c.), a special case of the more general unit of illumination, and "lumens per unit" (symbol E). The reader is then introduced to the ideas and importance of directions and "mean spherical" candle-power and colour temperature. Closely related to the subject of colour temperature is the spectral sensitivity of the human eye. The high sensitivity of the eye and also of the selenium (barrier layer) photoelectric cell for green, combined with the large proportion of this radiation normally available, is the reason why a wavelength of 0.55 μ is so popular. The green filter is not only very comfortable for long periods but the relatively short wavelength transmitted helps to procure the maximum possible resolution for an objective of given aperture. The advantages of photoelectric cells both of the vacuum and selenium or barrier layer pattern are fully discussed. The chief disadvantage is that the response to both the blue and the red is relatively too high. An extensive survey of illuminants, including daylight, is provided and many important tips are given such as the fact that the life of tungsten filament lamps may be prolonged if one or two low-voltage taps in the transformer are available. Thus a transformer nominally rated at 6 V may have additional terminals rated at 5, 4, and even 3 V. The life of a lamp when connected to the 5-V tapping is increased two to threefold and that on the 4-V tapping is almost unlimited. Projection lamps are an important type of illuminant: they range from the simple motor-car headlight, rated at some 24 W, to the multiple filament pattern with ratings of 50 to 500 W. In the ribbon filament type the spiral construction is avoided. Finally the illuminating system is discussed and consideration is given to the arrangement of the microscope substage and ancillary apparatus with low, medium, and high power, and high intensity illumination—the Köhler system.

This monograph is so full of important data that it might well find a place among those few books which the pathologist must keep within easy reach of his working bench.

G. M. FINDLAY.

Recent Advances in Pathology. By Geoffrey Hadfield and Lawrence P. Garrod. London. J. and A. Churchill. Fifth edition, 1947. Pp. 361. 60 illustrations. Price 21s. net.

Much new material has been fitted into the present edition without disturbing the plan or size of this well-known book in the "Recent Advances" series. Illustrations, mostly excellent photographs, are included where most needed. But in a subsequent edition a plate more in keeping with the others should be found to illustrate arsenical dermatitis and cancer (Fig. 9).

The chapter on nephritis, based as it is on the published work of Ellis, is greatly improved, and it is hoped that present concepts on renal damage may prove to be as near the truth as it is possible to get. Liver disease in the light of present knowledge is carefully reviewed, but the term "homologous serum hepatitis," though in common use, surely deserves a more appropriate synonym.

Much new work has been done on the relationship of enzymes and hormones to the cancer problem. Most of the available data has been carefully sifted and edited. The conclusion is drawn that malignant disease is due to intracellular disturbance, in nature chemical or even "microbic."

The chapter on ductless glands, contributed by Dr. E. F. Scowen, is a clear exposition of the facts. The author is at pains, and rightly, to differentiate between (1) hormones proper and (2) hormonally active substances recovered from the urine even though chemical constitution of the latter suggests a specific organ origin. It is not true to state that any given hormone is the prerogative of any one gland.

The subject index is carefully arranged and the name index should be a most valuable aid to bibliography.

MAGNUS HAINES.

We regret to announce the death of Dr. E. ff. Creed, Director of Pathology at King's College Hospital, London. An obituary notice will appear in the May issue.

J. clin. Path. (1948), **1**, 102.

ABSTRACTS

This section of the JOURNAL *is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this* JOURNAL *some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.*

BACTERIOLOGY

Isolation of Poliomyelitis Virus from the Throats of Symptomless Children. HOWE, H. A., and BODIAN, D. (1947). *Amer. J. Hyg.*, **45**, 219.

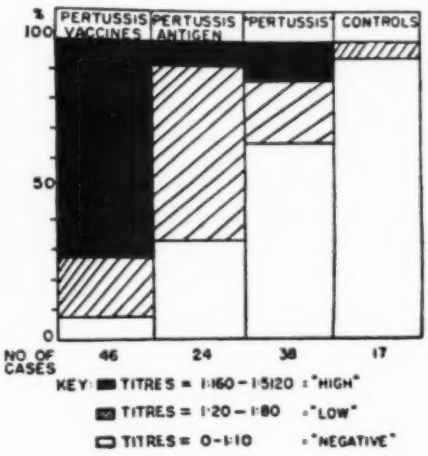
Poliomyelitis virus was isolated from 1 of 3 patients suffering from the disease and from 1 of 6 juvenile contacts but from none of 5 adult familial or 7 juvenile and 6 adult extrafamilial contacts of these cases. Virus was found in the throats of 2 out of 28 healthy children frequenting a neighbouring playground. One year later these children had developed antibodies to the virus isolated from them. *J. B. Ellison.*

An Outbreak of Diphtheria in a Highly Immunized Community. FANNING, J. (1947). *Brit. med. J.*, **1**, 371.

A small outbreak of diphtheria developed in a girls' school where 94% of the children had been immunized. Eight pupils developed diphtheria during the half-term holiday, but swabbing showed no carriers in the school itself. Two clinical cases later developed in non-immunized children, and after Schick-testing (read at 24 hours) 53% were recorded as Schick-positive and passively immunized by injecting 0.3 ml. of A.P.T. and 500 units of antitoxin. In spite of this 4 more cases developed, and reswabbing showed 8 carriers of *C. diphtheriae*. Further cases occurred during the next holiday, and 3 months after the onset of the epidemic swabbing of the school gave negative results. A further dose of A.P.T. was then given to all children. It is recommended that boosting doses of diphtheria prophylactic be given every 3 years during school life to maintain immunity.

The Agglutinative Reaction for Hemophilis Pertussis Following Whooping Cough and Following Immunization. DEGARA, P. F., and MAYER, S. A. (1947). *J. Pediat.*, **30**, 171.

In this investigation of agglutination reactions for *Haemophilus pertussis*, serum was collected from 130 children aged 1 to 12 years and comprising 4 groups: (1) children with a history of pertussis 3 months to 11 years previously; (2) children who had had a prophylactic course of pertussis vaccine 6 weeks to 9 years before; (3) children who had had a course of pertussis antigen, "tri-immunol"; (4) controls with no history of pertussis or prophylaxis. The results of the agglutination reactions in each group are shown in the diagram.



It is concluded that agglutination reactions are of limited value in determining the state of immunity of a child who is alleged to have had pertussis. Prophylactic vaccination is followed by a high agglutinin titre in a majority of cases. *M. MacGregor.*

Typhoid Fever in Vaccinated Laboratory Workers. HEADICKE, T. A. (1947). *J. infect. Dis.*, **80**, 113.

A brief account is given of four cases of mild and atypical typhoid fever in vaccinated laboratory workers engaged in typhoid research and the preparation of typhoid vaccine.

Diagnosis in each case was confirmed by positive blood culture. The agglutination results in two cases showed H-titres rising from zero to 320 and O-titres rising from zero to above 1,000. The inoculation history indicated that both patients had received full immunizing courses of

vaccine with booster doses within 6 months of the onset of the disease. After reviewing some recent literature on typhoid in inoculated individuals the author concludes that typhoid vaccine, where it does not prevent infection, so modifies the condition that the clinical manifestations are atypical and the course of the disease is mild except in massive infections. *H. J. Bensted.*

Human Glanders : Report of Six Cases. HOWE, C., and MILLER, W. R. (1947). *Ann. intern. Med.*, **26**, 93.

Six cases of laboratory workers engaged in research with *Malleomyces mallie* are reported. The incubation period was estimated at between 10 and 14 days, and the route of infection was probably the respiratory tract. Organisms were not recovered in spite of numerous attempts, but agglutination titres rose above 1 in 320 in 5 patients, and all 5 developed positive skin tests. The sixth case showed a rise of agglutination titre to 1 in 320 on one occasion only, the other tests being negative. Chemotherapy with sulphadiazine gave inconclusive results.

Ulcerative Colitis due to Fusospirochaetal Infection. (Colitis ulcerosa Fuso-spirochaetosa. Bericht über eine infektiöse Form der colitis ulcerosa im deutschen Konzentrationslager Sutthof b. Danzig in den Jahren 1943–45.) STARKUS, A. (1947). *Gastroenterologia, Basel*, **72**, 35.

The authors describe an acute fulminating form of ulcerative colitis with extremely high mortality. Bacteriological examination of the faeces showed spirochaetes of the Vincent type and fusiform bacilli. Vincent angina occurred simultaneously in a few cases. Necropsy of 130 cases showed haemorrhagic-diphtheritic inflammation with slough formation. In fulminating cases nearly the whole colon was haemorrhagic and swollen. But in a certain number the transverse colon remained free and the demarcation between the affected and non-affected parts was clear cut. Balantidia were found in one case; amoebae were not seen at all. Further proof as to the aetiology was obtained by treatment, as symptoms were usually cured by one injection of 0.3 to 0.5 g. neoarsphenamine. The whole epidemic was stopped in this way.

The author calls this clinical and pathological picture "colitis ulcerosa fuso-spirochaetosa," and considers that it should be distinguished from other types of ulcerative colitis.

The Intracutaneous Test in Cutaneous Leishmaniasis. DOSTROVSKY, A., and SAGHER, F. (1946). *Ann. trop. Med. Parasit.*, **40**, 265.

Parasites were detected in only 66.6% of patients diagnosed as suffering from cutaneous leishmaniasis, but in 261 infected persons an intradermal test was positive in 91.6%, while in 144 control patients presumed not to be specifically infected it was positive in only 6.3%. The reaction appeared to become positive within a few days of infection, and to remain so during the period of infection and for many years after the lesions had healed. Observations on 11 persons of the Prausnitz-Kuestner passive transfer test with the sera of 2 infected patients suggested that the reaction was of an allergic nature. *F. Murgatroyd.*

Serological and Clinical Observations in Cases of Epidemic Typhus in Sweden in 1945. (Serologiska och kliniska iakttagelser vid fall av epidemisk flackytyfus i Sverige 1945.) HAMMARSTROM, E., HELLSTEN, H., and FAHRAEUS, J. (1947). *Nord. Med.*, **33**, 700.

Serogical investigations by means of rickettsia agglutination and the Weil-Felix reaction were done on 104 cases of typhus and two control groups. The normal limits of agglutinating titre were taken as 1 in 320 for the Weil-Felix and 1 in 5120 as the upper limit of normal in the rickettsia agglutination test. A table showing the results in the 103 typhus cases suggests that the rickettsia agglutination is the more reliable diagnostic method.

Results Obtained by Streptomycin Treatment in 8 Cases of Diarrhoea with *Bacterium coli* and *Proteus* in the Stools. (Resultados obtenidos en 8 casos de diarrea con escherichia coli y proteus en las evacuaciones tratados con estreptomicina.) AGUIRRE, A., AGUAYO, A., and BENAVIDES, L. (1947). *Bol. méd. Hosp. infant.*, **4**, 4.

Colon bacilli were found in the stools of 8 cases of severe infantile diarrhoea. *Bacterium coli-gomez* and *Proteus* were each found in 3 cases. In 7 cases tested both organisms proved susceptible to streptomycin. Intensive treatment with sulphonamides and, in 4 cases, with penicillin failed. Symptomatic treatment was maintained during the whole course of the disease. Streptomycin was used after other drugs had failed. It was injected 3-hourly, the dose and length of administration varying from case to case. Six cases recovered and 3 died.

The authors recommend a dose of 100,000 to 150,000 units of streptomycin per kilo per day, according to the severity of the case.

Streptomycin in Miliary Tuberculosis. Its Effect on the Pathological Lesions of Generalized Miliary Tuberculosis in Human Beings. BAGGENSTOSS, A. H., FELDMAN, W. H., and HINSHAW, H. C. (1947). *Amer. Rev. Tuberc.*, **55**, 54.

Five patients who had miliary tuberculosis were treated with streptomycin. Although the cases proved fatal, it is encouraging that in 4 cases there was convincing evidence of regression and healing of miliary tubercles in the lungs, liver, and spleen, as shown by the occurrence of fibrosis and hyalinization and the absence of caseation. Tubercle bacilli were, however, usually recoverable from the lesions. A significant concentration of streptomycin was found in the cerebrospinal fluid, in contrast to its complete absence in the brain, which helps to explain the presence of active lesions in the brain with the absence of meningitis. On the other hand, no healing was observed in kidney or bladder lesions in spite of high streptomycin concentration. No histological evidence of any toxic effect of the drug could be found, with the possible exception of renal tubular damage in one case. *L. E. Houghton.*

Occurrence of Tuberculosis in B.C.G.-vaccinated Recruits. (Om förekomsten av tuberkulösa sjukdomar bland calmettevaccinerade värnpliktiga.) SAVILAHTI, M. (1947). *Nord. Med.*, **33**, 72.

Vaccination with B.C.G. on a large scale was first carried out in Finland during the war, when recruits were skin-tested and 13,400 negative reactors were vaccinated.

The incidence of all forms of tuberculous disease was observed over a period of 6 months to 2 years, and compared with that in a control series of unvaccinated subjects observed in 1933, while a more rigid control was obtained by a comparison of the incidence among subjects in the same call-up class who had given a positive reaction and hence were unvaccinated. Of the vaccinated subjects, 3% contracted tuberculosis within the 2-year period, one-half within the first 6 months, compared with 4% of the tuberculin-positive and 5.7% of the control group. The frequency of the various conditions is shown in the following table, the figures representing percentage incidence:

	Vaccinated subjects	Positive reactors	Controls
Pleural effusion	1.82	1.64	2.31
T.B. lymphadenitis ..	0.41	0.67	0.76
Erythema nodosum ..	0.09	0.06	0.07
Pulmonary tuberculosis	0.48	1.41	1.98
Other forms	0.14	0.06	0.20

Tuberculosis in vaccinated subjects was for the most part benign in character. The mortality figures per 10,000 were as follows:

	First year	Second year	Third year
Vaccinated	7.3	0	3.7
Positive reactors ..	4.6	8.5	6.5
Controls	12.2	19.2	15.7

D. J. Bauer.

B.C.G. Vaccination in Denmark in Recent Years. (B.C.G.-vaccinationen i Danmark i de senere Aar.) SLOTTVED, A. (1947). *Nord. Med.*, **33**, 68.

Whereas 24 B.C.G. vaccinations were carried out in Copenhagen in 1936, the figure had risen to 8,770 in 1945. The total figures for the whole of Denmark were 133 in 1936, rising to 30,311 in 1945; 93,139 vaccinations have been carried out in all since the service started. The percentage of the population vaccinated is greatest in Bornholm (23.3% by 1945); in other places it does not yet exceed 5%.

The incidence of bovine tuberculosis has been much reduced in Denmark in recent years. In 1937 50% of the population was free from tuberculosis; in 1946, 95 to 100%. In one district studied the percentage of positive tuberculin reactors was low in the age groups below 15 to 20 years, suggesting a reduced exposure during the last few years. *D. J. Bauer.*

Rheumatoid Arthritis. IV. Hemolytic Streptococcus Precipitin Reactions. WALLIS, A. D. (1947). *Amer. J. med. Sci.*, **213**, 87.

The author concludes that it is unlikely that the sera of patients with rheumatoid arthritis contain a genuine excess of haemolytic streptococcus precipitins. The antigens employed in the precipitin reactions were: (1) extracts made by Lancefield's method from haemolytic streptococci of groups A, B, C, D, E, F, and G, and (2) the C-carbohydrate substance characteristic of group A. The sera were obtained from patients with typical rheumatoid arthritis and from non-arthritic individuals free from recent streptococcal infection.

T. D. M. Martin.

The Relation Between Induced Resistance to Penicillin and Oxygen Utilization. BELLAMY, W. D., and KLIMEK, J. W. (1947). *J. Bact.*, **53**, 374.

After 64 transfers a strain of *Staphylococcus aureus* had its resistance to penicillin increased over 60,000. This strain also lost its ability to grow anaerobically while its rate of aerobic growth was from one-half to two-thirds that of the penicillin-sensitive parent strain. Under similar conditions three other organisms, *Streptococcus faecalis*, *Str. agalactiae*, and *Clostridium perfringens* developed very slight resistance to penicillin; the resistance of *Str. faecalis* was increased only 11 times in 47 transfers, that of *Str. agalactiae* 6 times in 24 transfers, and that of *Cl. perfringens* 10 times in 25 transfers. It is suggested that penicillin interferes with some anaerobic metabolism. Organisms which are unable to develop or utilize an alternative energy mechanism do not become resistant to penicillin.

G. M. Findlay.

A Mechanism for the Development of Resistance to Streptomycin and Penicillin. KLEIN, M. (1947). *J. Bact.*, **53**, 463.

The actual number of bacteria employed was found to play an important part in determining the minimum and maximum effective doses of penicillin or streptomycin on any particular organism. By the passage of organisms through a medium containing progressively larger doses of the antibiotic, resistance to both penicillin and streptomycin was raised. But whereas resistance to streptomycin increased very rapidly to a high level, increased penicillin resistance developed much more slowly and at a lower level. Variants possessing very high resistance to penicillin were not found in the investigation reported. *H. J. Bensted.*

Penicillin Therapy of Subacute Bacterial Endocarditis. A Study of the End Results in Thirty-four Cases, with Particular Reference to Dosage, Methods of Administration, Criteria for Judging Adequacy of Treatment and Probable Reasons for Failures. PRIEST, W. S., SMITH, J. M., and MCGEE, C. J. (1947). *Arch. intern. Med.*, **79**, 333.

Of 34 patients with subacute bacterial endocarditis 22 are alive and free from evidence of disease 13 to 35 months after completing a course of penicillin treatment. Great stress is laid on the importance of adequate dosage during treatment, 500,000 units given intravenously being the daily minimum recommended. Larger doses were employed in some cases. Daily doses of less than 400,000 units are useless. Treatment was continued for over 4 weeks; if at the end of this time the patient was not improving the dose was increased to 2,000,000 units daily.

Alan Kekwick.

The Cellular Mechanism of Recovery after Treatment with Penicillin. I. Subacute Bacterial Endocarditis. MOORE, R. A. (1946). *J. Lab. clin. Med.*, **31**, 1279.

Lesions of the heart in subacute bacterial endocarditis in 22 patients treated with penicillin and in 8 patients given no specific therapy are compared. The vegetation

is composed of a central core of necrotic tissue, a layer of bacterial colonies embedded in fibrin, and a superficial thin layer of fibrin. Penicillin promotes healing in subacute bacterial endocarditis, but the basic processes of healing are not modified. These processes are: covering of the exposed surface of the vegetation with fibrous tissue, invasion of the layer of colonies and phagocytosis of bacteria, calcification of bacterial colonies, hyalinization and calcification of the central core of the vegetation, and endothelization of the spaces and clefts in the vegetation. In some patients healing is accompanied by excessive calcification and the result is a calcified stenosis of the valve. Healing in subacute bacterial endocarditis is essentially the conversion of a vegetative endocarditis into a chronic endocarditis characterized by superficial vascularized connective tissue and central nodular calcification. *T. Semple.*

Enhancement of Plasma Penicillin Concentrations by Caronamide and Sodium Benzoate. STRAUSS, E., RICHBURG, P. L., SABA, P. Z., and ALEXANDER, J. E. (1947). *J. Lab. clin. Med.*, **32**, 818.

In this study 35 subjects received caronamide by mouth together with penicillin (*a*) by mouth, (*b*) by intramuscular injection in aqueous solution, and (*c*) by intramuscular injection in peanut oil and beeswax. A study of the effectiveness of caronamide and sodium benzoate, individually and in combination, was carried out in 8 additional subjects. When given by mouth, the combination of both caronamide and sodium benzoate resulted in a marked enhancement of plasma penicillin concentrations and yielded better results than either drug alone. The only evidence of toxicity noted was gastro-intestinal intolerance after 12 g. of caronamide by mouth in 24 hours. For the intramuscular tests the sodium salt of amorphous penicillin was used. In nearly all cases plasma penicillin concentrations were higher when caronamide was administered concurrently. The effect of 2 injections at 4-hourly intervals and of large single intramuscular injections of penicillin was not augmented by the caronamide. Sodium benzoate in 2-g. doses enhanced the plasma penicillin concentrations after multiple injections in a manner similar to that of caronamide. Results with the calcium salt of amorphous penicillin (300,000 units per ml.) in peanut oil and beeswax indicated that caronamide (1.5 g. by mouth every 2 hours for 24 hours) gave erratic augmentation effects, but in the majority of instances plasma penicillin concentrations were increased. *G. B. West.*

The Role of Coagulase in Staphylococcal Infections. SMITH, W., HALE, J. H., and SMITH, M. M. (1947). *Brit. J. exp. Path.*, **28**, 56.

The association of the pathogenicity of staphylococci with their coagulase activity was suggested by circumstantial evidence and by *in vitro* investigation of phagocytosis. The authors now report investigation of this association *in vivo*. It is concluded that staphylococcal coagulase is an important factor in the pathogenicity of staphylococci, though not the only, or even the most important, factor, because it can have no direct part in the cellular necrosis occurring in characteristic focal lesions, apparently due to toxin. But toxin cannot be produced until the organism has gained a foothold, and for this, in natural infections with small numbers of cocci, coagulase production is essential. The latter may also be important at later stages in overcoming the phagocytic response and in aiding the setting up of metastatic infection by infected emboli. There seems little prospect of immunity against staphylococci being conferred by measures directed against coagulase activity, since coagulase itself is non-antigenic. The isolation and chemical definition of coagulase are urgently necessary. *G. T. L. Archer.*

Mycosis of the Nervous System. (Micoses do sistema nervoso.) LACAZ, C. DA SILVA, DE ASSIS, J. LAMARTINE, and BITTENCOURT, J. M. TAQUES (1947). *Arch. Neuro-psiquiat.*, **5**, 1.

Neuromycotic lesions can be placed in two groups—those in which there is "tumour" formation (abscess, cyst, granuloma) and those in which there is meningitis. Both forms often occur simultaneously. In view of the ambiguity of clinical findings and of findings in the cerebrospinal fluid and blood, diagnosis can rest with certainty only on the actual identification of the organism or culture. *J. J. Keevil.*

Use of Papain in Cultivation of Bacteria from the Blood. DREYFUSS, F. (1947). *Amer. J. clin. Path.*, **17**, 365.

A papain-broth medium is recommended for blood cultures. The preparation of the medium is simple. It is claimed that it combines many of the advantages of saponin and trypsin; in the author's hands it appeared useful in isolating streptococci, especially of the viridans type. The growth of micro-aerophilic organisms is also favoured. Papain-broth serves as an anticoagulant, is shown to inhibit action of sulphonamides and penicillin (the latter because of a contaminating subtiloid bacillus contained in the dry powder), and has complement-fixing properties, thereby enhancing the growth of organisms. Because of its fibrinolytic action it can be employed with advantage when clot cultures are to be made. *R. Salm.*

The Evaluation of Culture Mediums for the Routine Isolation of the Gonococcus. THYER, J. D., SCHUBERT, J. H., and BUCCA, M. A. (1947). *J. vener. Dis. Inform.*, **28**, 37.

A comparison is made of the efficacy of four different culture media: (1) "difco" chocolate-agar prepared with "bacto-proteose" peptone, No. 3 agar, and "bacto-haemoglobin"; (2) Peizer's horse plasma-haemoglobin agar; (3) Mueller-Hinton starch agar, as described by the authors; (4) a modification of McLeod's medium in which McLeod's phosphate-infusion agar was used as a base, enriched with plasma-haemoglobin in the same proportions as described by Peizer. The presence of the gonococcus was confirmed by Gram's stain and the sugar fermentation and oxidase tests.

With each medium 115 cultures were prepared; 95.7% of positive results were obtained with the special medium, 87.0% with the Peizer medium, 79.3% with the difco medium, and 63.5% with that described by Mueller. On 87 comparable plates in each group, each containing 300 or fewer colonies, the average number of colonies was 58.8 for the special, 39.6 for the Peizer, 37.9 for the difco, and 10.2 for the Mueller medium. *R. R. Willcox.*

BIOCHEMISTRY

Elimination of Urea, Sodium, and Chlorine in the Supersaturated Urine and According to the Intensity of Diuresis. (Las eliminaciones de urea, sodio y cloro por la orina con sobrecargas, y según la intensidad de la diuresis.) CANTERA, M. T., JIMÉNEZ DÍAZ, C., and MERCHANTE, A. (1947). *Rev. clín. esp.*, **24**, 9.

Ambard's view that urea and sodium chloride can simultaneously reach independent concentrations in urine in case of supersaturation, with dearth of water intake, is questioned. The osmotic pressure of total solids must be taken as the criterion of renal capacity, and the idea seems surprising that maximal concentration of sodium chloride should not hamper the elimination and concentration of other elements like urea. If the kidney cannot exceed a certain limit of osmotic concentration this must be so. Some recent workers have held that in the conditions mentioned chlorides and urea may vary inversely.

The authors studied this problem in normal human subjects given varying amounts of water and a constant low-nitrogen diet and ingesting excess of urea or sodium chloride. They conclude that increased ingestion of sodium chloride with low water intake causes increase of urinary volume at the cost of water from the tissues, with increased elimination of sodium and chlorine, but with retention of a part in order to economize water. There is a well-marked diminution in the output of urea. The reverse holds when urea is given in excess. The sodium-chloride concentration at a given osmotic level can only increase at the cost of urea. There is a reciprocal inverse ratio. When enough water is ingested excess of the various salts is eliminated without bringing this function into play. The saline elimination with small amounts of water is the chief determining factor of urinary volume. In certain conditions the sodium and chlorine may be unequally excreted as in pathological states, and as seen from the action of suprarenal cortex.

D. T. Barry.

Water and Salt Depletion. MARRIOTT, H. L. (1947). *Brit. med. J.*, **1**, 245, 285, and 328.

Cases of water depletion are classified into three grades: (1) *Early.*—Definite thirst is the only sign; the deficit is about 2% of the body weight, equivalent to 1.5 litres in a 70-kg. man. (2) *Moderately severe* (3 to 4 days without water).—Marked thirst, oliguria, weakness, and slight personality changes are noted, but the subject is still capable of fair effort; the deficit is about 6% of body weight (4.2 litres in a 70-kg. man). (3) *Very severe.*—Here in addition there is a marked impairment of mental and physical capacity; the deficit ranges from 7 to 14% of body weight, or 5 to 10 litres in a 70-kg. man.

The author divides the degree of salt depletion into three grades: (1) Slight to moderate, with absence of chloride in the urine (except in Addison's disease), indicating a deficit of up to 0.5 g. sodium chloride per kilo body weight; this represents a deficit of up to 4 litres of isotonic saline in a 70-kg. man. (2) Moderate to severe depletion, absence of chloride in the urine with anorexia but with a sytolic pressure above 90 mm. Hg and a deficit of 0.5 to 0.75 g. salt per kilo body weight, equivalent to a deficit of 4 to 6 litres of isotonic saline. (3) Severe to very severe depletion, absence of chloride in the urine, with apathy, stupor, and vomiting, and a systolic blood pressure below 90 mm. Hg; suggesting a deficit of 0.75 to 1.25 g. per kilo body weight, or 6 to 10 litres of isotonic saline in a 70-kg. man. The author recommends the use of a quantitative test described by Fantus (*J. Amer. med. Ass.*, 1936, **107**, 14).

In patients with inefficient kidneys incapable of excreting a concentrated urine, the daily urinary output may need to be not less than 1.5 litres. A period of 24 hours is too long for a review of output; chloride content and volume should be reported 8-hourly; the aim should be an excretion of 3 to 5 g. sodium chloride per litre in the adult and 570 ml. urine per 8 hours. Attention is called to the correct treatment of established deficiencies. In pure water depletion patients require water only, and particularly is this the case in infants whose kidneys cannot deal with a superfluity of salt. In salt depletion the wrong treatment consists chiefly in failure to give saline and the patient dies of oligaemic circulatory failure. Administration of water beyond that required to cover unavoidable water losses may have serious consequences. In collapse with the pylorus closed, water fills the stomach and produces vomiting, which may drown the patient whose cough reflex is gravely depressed. In salt depletion isotonic saline should be given intravenously, but as soon as chloride appears in the urine only hypotonic saline is required—0.425% for adults, 0.2% for infants—until the salt balance appears to be fully restored as indicated above in an 8-hourly period. In mixed salt and water depletion hypotonic saline is needed. In marked peripheral circulatory failure in adults the first pint should be given in 10 minutes, a second pint in 15 minutes, a third pint in 20 minutes, a fourth pint in 30 minutes, and a pint every 2 hours until blood pressure is restored to normal.

C. C. N. Vass.

Investigations of the Cerebrospinal Fluid in Cases of Rheumatoid Arthritis. SUNDELIN, F. (1947). *Amer. J. Med.*, **2**, 579.

In 141 cases of rheumatoid arthritis (38 male and 103 female) slight changes in the cerebrospinal fluid were found in the globulin-albumin quotient in 52 cases, although no considerable difference appears in total protein values. Fifteen abnormal colloid gold curves were also obtained, and 7 increased cell counts. All the patients with pathologically increased protein contents, except 1, had neurological symptoms.

Excretion of 17-Ketosteroids in Ankylosing Spondylarthritis and in Rheumatoid Arthritis: A Preliminary Report. DAVISON, R. A., KOETS, P., and KUZELL, W. C. (1947). *J. clin. Endocrinol.*, **7**, 201.

An investigation of the 17-ketosteroid output in the urine of patients with ankylosing spondylitis is described. In 13 male patients an average excretion of 27.3 mg. in 24 hours was found, with a range of from 19.2 to 43.7. The average value is somewhat high for the group (the average for normal males is 14 mg.). This is contrasted with the findings in 11 female patients with rheumatoid arthritis who excreted an average of 12.8 mg. in 24 hours, with a range of from 3.5 to 21.6 (average for normal females 10 mg.).

E. F. Scowen.

An Evaluation of the Urethral Smears as an Index of Androgenic Deficiency in the Male. COHEN, E. J. (1947). *J. clin. Endocrinol.*, **7**, 186.

The technique of studying urethral smears in the male is described; the findings in 15 normal and 15 hypogonadal male patients were examined. No significant difference was observed between the two groups. In the normal male the administration of oestrone sulphate and the injection of oestradiol benzoate, sufficient to produce oligospermia and lowering of the 17-ketosteroid output, did not affect the urethral smear.

E. F. Scowen.

A Rapid Supra-vital Staining Method for Assessing the Viability of Human Spermatozoa. CROOKE, A. C., and MANDL, A. M. (1947). *Nature, Lond.*, **159**, 749.

A method of assessing viability of human spermatozoa is described. Four or 5 drops of seminal fluid are well mixed with 1 drop of the supravital stain (50 ml. distilled water + 1 g. "revector soluble blue 706" + 1.5 g. glucose + 0.1 g. sodium chloride + 0.3 g. disodium hydrogen phosphate, hydrated + 0.005 g. potassium dihydrogen phosphate, anhydrous; the solution must be freshly prepared or made up in 1-ml. ampoules and autoclaved). The mixture is left for 2 to 3 minutes. The spermatozoa remain motile in this stain as long as they do in seminal fluid. A smear made from one drop of the mixture is dried in air and fixed in alcoholic mercuric chloride (half volume of saturated $HgCl_2$ in distilled water + half volume of absolute alcohol) for 15 to 20 seconds, dipped into alcoholic iodine (90% alcohol containing enough iodine in potassium iodide to make a straw-coloured solution) and then into 90% alcohol and allowed to dry. It is counterstained with 1% neutral red in distilled water for 15 to 30 seconds and differentiated carefully in 90% alcohol. The smear may then be examined directly by oil immersion or passed through absolute alcohol and mounted in Canada balsam.

The nuclei of dead spermatozoa stain blue or purple, while those of living spermatozoa are clear red.

Peter C. Williams.

A Study of the Metabolism in Addison's Disease. I. On Carbohydrate and Phosphorus Metabolism. [In English.] HELVE, O. (1947). *Acta med. scand.*, **127**, 543.

The present investigations were carried out in 32 cases of Addison's disease and 40 controls. The fasting blood sugar in all 32 cases showed no significant deviation from the normal, and treatment with desoxycorticosterone acetate and a low potassium diet or by high sodium-chloride intake was without obvious effect on the blood sugar. A glucose-tolerance test was performed on 23 cases; its results in all were within normal limits, and in 10 cases the adrenaline-tolerance test showed little if any deviation from the normal controls.

An investigation of the total blood phosphorus and of its component fractions revealed a slight rise in inorganic phosphate, in spite of a suggestion of a fall in the total phosphorus in 6 patients. Treatment with desoxycorticosterone acetate did not affect this, and the blood-phosphorus changes in response to the administration of glucose, adrenaline, and insulin were essentially the same as in the controls.

E. F. Scowen.

Distribution of Cholesterol, Cholesterol Esters and Phospholipid Phosphorus in Blood in Thyroid Disease. FOLDS, F. F., and MURPHY, A. J. (1946). *Proc. Soc. exp. Biol., N.Y.*, **62**, 218.

The plasma cholesterol, plasma cholesterol ester, and plasma phospholipid phosphorus were found to be significantly increased in hypothyroid conditions. The lipid ratios are also altered. The plasma cholesterol ester/total cholesterol ratio and the plasma cholesterol/plasma phospholipid phosphorus ratio were significantly increased. The cell cholesterol/plasma cholesterol and the cell phospholipid phosphorus/plasma phospholipid phosphorus ratios were decreased. These values returned to normal with adequate treatment. The changes in hyperthyroidism were inconstant, the plasma phospholipid phosphorus being the only constituent which showed a significant decrease. In both hypo- and hyperthyroidism no significant change was demonstrated in the cell lipid values.

E. F. Scowen.

Investigations of some Biochemical Changes Occurring during Treatment of Hyperthyroidism. [In English.] LUNDBAEK, K. (1947). *Acta med. scand.*, **127**, 193.

This paper describes a series of investigations carried out during the treatment of 6 cases of hyperthyroidism with methyl thiouracil. A study was made of the basal metabolic rate (B.M.R.); the excretion of nitrogen, creatine, sodium, chloride, and calcium; the cell volume; serum protein, cholesterol, and uric acid. The fall in the B.M.R. began at once and continued in an almost straight line. Increase in weight occurred at a variable time and was not related to B.M.R., pulse rate, or serum cholesterol. The serum cholesterol was shown to be an unexpectedly valuable index of individual thyroid function, with a very close relation to the pulse rate and a less close relation to the B.M.R. and weight. The serum uric acid showed no uniform change, and changes in cell volume and serum protein were transient. There was an even fall in nitrogen excretion. No variation was seen in creatinine excretion. A decrease in calcium excretion occurred after a latent period of a week, but there was no constant relation to thyroid function. The effects of treatment on sodium and chloride excretion varied from patient to patient. No uniform changes were observed in the volume of urine secreted.

Raymond Greene.

The Effect of Oral Thyroid Medication upon the Prothrombin Time. SHAPIRO, S. (1946). *J. clin. Endoc.*, **6**, 742.

The plasma prothrombin time was employed as a test of hepatic function in patients receiving thyroid preparations by mouth. In 16 patients taking 0.1 to 1 g. of thyroid globulin daily for periods varying from 10 to 104 days no increase in prothrombin time occurred.

R. Bodley Scott.

Study of Carbohydrate Metabolism of the Newborn and Infants on the Basis of Double Glucose and Galactose Tolerance Tests. (Beiträge zum Zuckerstoffwechsel Neugeborener und Säuglinge auf Grund Doppelbelastungen mit Glucose und Galactose.) BARTA, L., and SASS-KORTSÁK, A. (1947). *Paediat. danub.*, **1**, 88.

This article records an interesting and probably unique series of sugar tolerance tests with glucose and galactose

on newborn infants, some within 12 hours of birth, and on infants from 1 to 20 months old. In the majority the results were very similar to the adult response. Galactose was usually perfectly tolerated, no increase occurring in the blood after 6 to 10 g. had been given; glucose was sometimes less perfectly tolerated. A second dose of the sugars, to evoke a Staub-Traugott reaction, gave irregular results. It is suggested that abnormally high curves are due to "functional lability" of the liver.

R. D. Lawrence.

Carbohydrate Metabolism in the Coeliac Syndrome. EMERY, J. L. (1947). *Arch. Dis. Childh.*, **22**, 41.

The absorption of glucose has been studied in 13 cases of the coeliac syndrome and controls. In the coeliac syndrome the oral glucose tolerance curve was flat, but it was more normal if larger doses of glucose were ingested. After insulin injection the immediate fall in blood sugar was similar in the coeliac and control groups, but recovery was usually delayed in the coeliac cases. The recovery rate was not affected by administration of adrenaline, but was improved by the ingestion of glucose. The administration of adrenaline caused a rise in blood sugar, which was less than normal in the coeliac group unless glucose was simultaneously administered by mouth, when an immediate and rapid rise in blood sugar occurred in both groups. The administration of adrenaline after insulin produced little effect on the blood sugar in the coeliac patients studied. The intravenous glucose tolerance tests were within normal limits in all cases.

It is concluded that glucose absorption occurs normally in the coeliac syndrome, on the basis of the improvement in the oral glucose tolerance curve with increasing dosage of glucose and the response to oral glucose during insulin hypoglycaemia. The normal response following glucose and adrenaline indicates that the rate of absorption is normal. The flat oral glucose tolerance curve is attributed to more rapid fixation of glucose, presumably in the liver, since the intravenous glucose tolerance curves were normal. The possibility that the underlying fault may be a deficiency of liver glycogen is discussed.

A. C. Frazer.

Mauriac's Syndrome (Retardation of Growth with Hepatomegaly and Disturbance of Fat Distribution in the Diabetic Child) and its Relation to Von Gierke's Disease. (Le syndrome de Mauriac retard de taille avec hepatomégalie et troubles de la répartition des graisses chez l'enfant diabétique et ses rapports avec la maladie glycogénique de Van Creveld-van Gierke.) HOUET, R. (1947). *Ann. Pædiat., Basel*, **168**, 113.

This paper deals with the syndrome first described by Mauriac. Comparisons are drawn between this condition and von Gierke's syndrome, a type of hepatomegaly described a few years before Mauriac's original communication. In Mauriac's syndrome, as the present author admits, biochemical changes are not very numerous or consistent, and much further investigation is needed. Nevertheless, it seems fairly clear that the biochemistry in this group is quite different from that of von Gierke's disease and resembles that of the true diabetic.

No definite conclusion is reached regarding the aetiology. The author thinks that the problem is likely to remain unsolved until the disease can be reproduced in animals.

Patrick Mallam.

Spontaneous Hypoglycemia with Special Reference to the Diagnosis of Hyperinsulinism. [In English.] LUFT, R. (1947). *Acta med. Scand.*, **127**, 65.

Fifteen normal subjects underwent a 3-hour intravenous glucose tolerance test and an intravenous insulin tolerance test, and 32 subjects the Exton-Rose two-dose 1-hour glucose tolerance test; the limits of the normal were thence defined in terms of mean and standard deviation for each sampling time.

Six patients with spontaneous hypoglycaemia were studied by these tests; of these, 2 had an islet-cell adenoma of the pancreas, and 1 an islet-cell carcinoma with many metastases; in the other 3 "reactive" hyperinsulinism was considered to be present. In all cases the intravenous glucose tolerance test gave low values, and hypoglycaemic symptoms appeared within 90 minutes of the start. In the intravenous insulin tolerance test the 3 organic cases showed low values of blood sugar, which failed to rise towards fasting level; the 3 "reactive" cases gave results within the normal limits, though all 6 showed marked symptoms of hypoglycaemia.

G. Discombe.

Chronic Subclinical Impairment of the Liver. Early Diagnosis and Treatment. Further Improvement and Evaluation of Certain Liver Function Tests. MATEER, J. G., BALTZ, J. I., STEELE, H. H., BROUWER, S. W., and COLVERT, J. R. (1947). *J. Amer. med. Ass.*, **133**, 909.

These authors describe eleven different liver function tests and give what they consider normal values for them. It is claimed that by using the three most delicate tests (cephalin flocculation, thymol turbidity at *p*H 7.55, and a new bromsulphthalein test) cases of subclinical liver damage can be detected. The authors indicate which combinations of tests they advise in various types of liver disease.

The detection of subclinical liver damage is regarded as important, for these workers maintain that the treatment of such cases with a high-carbohydrate, high-protein, low-fat diet (C 350 to 400 g.; P 125 to 150 g.; F 35 g.) with added vitamins (A and B complex) causes the sensitive tests to return to normal and prevents the development of permanent damage or cirrhosis.

C. Hardwick.

Pancreatic Secretion in Hepato-biliary Diseases. (La sécrétion pancréatique au cours de certaines affections hépato-biliares.) HERFORT, K. (1947). *Gastroenterologia, Basel*, **72**, 51.

Chronic pancreatitis is usually secondary to gall stones or cholecystitis. The author studied the pancreatic secretion in cases of liver damage caused by cirrhosis, infective hepatitis, and acute atrophy of the liver. He had previously examined the external secretion of the pancreas in 60 cases of "cholecystopathy." In 60% of the cases involvement of the pancreas manifested itself clinically and in laboratory tests. In one-quarter of his cases he could not prove involvement of the pancreas. The participation of the pancreas in liver affections has not been much studied.

In 20 patients with cirrhosis, 12 males and 8 females, examination of pancreatic external secretion showed that lipase is the enzyme whose secretion is first diminished much before that of trypsin. Lack of lipase could be

shown by examination of the faeces in 3 cases only. It is well known that the splitting of fats can occur through action of intestinal bacteria to such a degree that no deficiency of pancreatic lipase in the faeces can be detected, although disturbance of the pancreatic secretion can be proved. In 9 out of 20 cirrhotic patients pancreatic secretion was seriously disturbed. The deficiency of lipase was most marked, in contrast with the even decrease in pancreatic enzymes in cases of tumour. The degree of the pancreatic insufficiency is not parallel with the severity of the cirrhosis.

Twenty-three cases of infective hepatitis were examined; 8 showed disturbed pancreatic function, 4 had considerable insufficiency after the disappearance of the jaundice. Two cases of acute yellow atrophy of the liver after cinchophen administration were examined. The secretion of duodenal juice was normal after secretion injection. Both patients died and the histology of the pancreas showed no abnormality. *E. Forrai.*

Observations on the Diurnal Excretion of Urobilinogen in the Urine of Normal Subjects and of Patients with Laënnec's Cirrhosis. PELLEGRINO, E., PATEK, A. J., COLCHER, A., and DOMANSKI, B. (1947). *J. Lab. clin. Med.*, **32**, 397.

Urobilinogen excretion was determined at three periods in the day in 39 healthy subjects and in 36 patients with cirrhosis of the liver. The mean urobilinogen excretion per hour in the urine was 0.3 Ehrlich units for normal subjects and 0.6 units for patients with cirrhosis. The period of maximum excretion varied in different subjects and in the same subject at different times. There was poor correlation between the degree of urobilinogen excretion and the clinical severity of the liver disease.
R. B. Lucas.

The Assessment of Liver Damage Following Trichlorethylene and Di-ethyl Ether Anæsthesia. ARMSTRONG, D. M. (1947). *Anæsthesia*, **2**, 45.

Assessment of liver damage by the cephalin-cholesterol test showed severe damage by di-ethyl ether and less damage after trichlorethylene anaesthesia.

Biochemical Studies During Malarial and Artificial Fevers. GALL, E. A., and STEINBERG, A. (1947). *J. Lab. clin. Med.*, **32**, 508.

Preliminary studies on 18 malarial patients had shown the development of a transient hypophosphataemia during febrile paroxysms. Hyperglycaemia is first produced by the accelerated metabolism during fever. Hexosephosphate is formed and deposited in the tissues, thus causing hypophosphataemia. At the end of the febrile episode the hexosephosphate dissociates and the amount of inorganic phosphorus in the serum returns to normal. *J. E. Page.*

Postacidotic State of Infantile Diarrhea : Symptoms and Chemical Data. Postacidotic Hypocalcemia and Associated Decreases in Levels of Potassium, Phosphorus and Phosphatase in the Plasma. RAPOPORT, S., DODD, K., CLARK, M., and SYLLM, I. (1947). *Amer. J. Dis. Child.*, **73**, 391.

A report is made on the signs, symptoms, and chemical changes in infants with diarrhoea during acidosis and after recovery from acidosis. The authors recognize two phases in the illness: "(1) the phase of acidosis and dehydration, during which losses of extracellular and intracellular ions and fluids occur, and (2) the post-acidotic phase, during which, after correction of deficits of extracellular ions, depletion of the nonextracellular ions ensues because of avid uptake of these ions by soft tissues and bone. Plasma depletion of these ions is attended by pronounced signs and symptoms."
Eveline A. Bishop.

HAEMATOLOGY

Folic Acid in Pernicious Anaemia. Its Effect as Shown by Serial Sternal Punctures. LEVY, H. (1947). *Brit. med. J.*, **1**, 412.

Serial marrow punctures on a patient with Addisonian pernicious anaemia revealed as rapid a reversion to normoblastic erythropoiesis as has been shown to occur after liver therapy. Abnormal granulopoiesis persisted for at least 48 hours after the beginning of treatment.

Relative Clinical and Hematologic Effects of Concentrated Liver Extract, Synthetic Folic Acid and Synthetic 5-Methyl Uracil in the Treatment of Macrocytic Anemias in Relapse. FROMMEYER, W. B., and SPIES, T. D. (1947). *Amer. J. med. Sci.*, **213**, 135.

An important paper. The authors compare the therapeutic effects of concentrated liver extract, synthetic folic acid, and 5 methyl-uracil, using the same patients in successive relapses. Liver extract seemed to be slightly more effective than folic acid; 5 methyl-uracil was much less useful.

Nonutilization of Conjugated Folic Acid in Pernicious Anemia. HEINLE, R. W., NELSON, E. M., NELSON, H. V., and WELCH, A. D. (1947). *J. Lab. clin. Med.*, **32**, 336.

Folic acid conjugate (heptaglutamate) was ineffective when administered to three patients with Addisonian anaemia. Two of the patients subsequently responded to synthetic folic acid.

The Treatment of Pernicious and Related Anemias with Synthetic Folic Acid. I. Observations on the Maintenance of a Normal Hematologic Status and on the Occurrence of Combined System Disease at the End of One Year. VILTER, C. F., VILTER, R. W., and SPIES, T. D. (1947). *J. Lab. clin. Med.*, **32**, 262.

This work provides further evidence that folic acid is ineffective in controlling the neurological complications of Addisonian pernicious anaemia, and that reliance upon it in the maintenance treatment of this disease is to be deprecated. It confirms the view, however, that folic acid is effective as a haematinic in megaloblastic anaemia, and that it may be of definite clinical value in cases associated with intestinal deficiency.
L. J. Davis.

Atypical Anemia, with Spherocytes and Target Cells Coexisting in the Blood. DISCOMBE, G., and WATKINSON, G. (1947). *Amer. J. med. Sci.*, **213**, 153.

An interesting report.

A Study of the Sternal Marrow and Peripheral Blood of Fifty-five Patients with Plasma Cell Myeloma. DIGGS, L. W., and SIRRIDGE, M. S. (1947). *J. Lab. clin. Med.*, **32**, 167.

Stilbamidine and Pentamidine in Multiple Myeloma. SNAPPER, I. (1947). *J. Amer. med. Ass.*, **133**, 157.

Stilbamidine and/or pentamidine administered intravenously resulted in clinical remission with alleviation of pain. The course of the disease appeared to be checked. These substances form compounds with ribonucleic acid in the cytoplasm of the myeloma cells. Deeply basophilic inclusions develop.

The treatment was combined with a diet low in protein by American standards: the specimens given provide about as much protein as the current British rations.

Icterus Neonatorum : Its Incidence and Cause. FINDLAY, L., HIGGINS, G., and STANIER, M. W. (1947). *Arch. Dis. Childh.*, **22**, 65.

The authors conclude that "physiological" jaundice in the newborn is more likely to be due to hepatic immaturity than to haemolysis.

The Influence of Heat and Formalin Upon the Rh Agglutinogen. LUBINSKI, H. H., and PORTNUFF, J. C. (1947). *J. Lab. clin. Med.*, **32**, 178.

After 5 to 20 minutes heating at 50° C. red blood cells are not, or hardly, agglutinated by anti-Rh agglutinating and blocking sera, but normal agglutination occurs with anti-A, anti-B, anti-M, and anti-N sera. Agglutination of red-cell suspension, after treatment with dilute formalin (0.1 to 1%), is inhibited to a greater extent for anti-Rh serum than for anti-A and anti-B sera of equal titre. This latter effect is not due to haemolysis of the red cells. The reason may be due to: (1) the Rh agglutinogen is situated on the surface of the cells and the others within the cells; (2) the Rh agglutinogens may be less numerous and are, therefore, destroyed more quickly; or (3) there may be a difference of chemical structure of the different agglutinogens. *John F. Wilkinson.*

An Anticoagulant Present in the Blood of a Clinically Haemophiliac Patient. (Sur un anticoagulant présent dans le sang d'un sujet cliniquement hémophile.) LAMY, M., BURSTEIN, M., and SOULIER, J. P. (1946). *Rev. hémat.*, **1**, 421.

In a patient suffering from the clinical manifestations of haemophilia the blood plasma delayed the clotting of normal blood or plasma. Examination showed normal bleeding time, tourniquet test, capillary fragility, blood count, platelet count, prothrombin time, clotting on addition of thrombin, and content of "masked" antithrombin; the anti-fibrinolytic power of the blood was normal. When 1/10 to 1/20 volume of the patient's non-citrated plasma, unheated or heated to 65° C. for 30 minutes, was added to normal blood or to normal plasma, which was then recalcified, the clotting time was prolonged 2 to 18 times. The clotting time of the patient's recalcified citrated plasma varied with the content of platelet substance: it was greatly prolonged when plasma freed of platelets by centrifugation and filtration was used, less prolonged when platelets were normal in number and intact, and least (though still greater than normal) when the platelets were destroyed by freezing or by addition of distilled water; it was not affected by addition of protamine.

The authors conclude that a circulating anticoagulant was present; it was neither heparin, an anti-prothrombin, nor an anti-thrombin; it probably interfered with the activation of the thromboplastin of plasma or platelets. *G. Discombe.*

MORBID ANATOMY AND HISTOLOGY

Further Notes on the Pathology of Acute Epidemic Hepatitis and Homologous Serum Jaundice. WOOD, D. A., and BLACK, M. B. (1946). *Amer. J. clin. Path.*, **16**, 746.

The pathology was studied at necropsy in 8 patients dying from 2 to 10 days after the onset of hepatitis, most of the cases being of homologous serum jaundice. On the second day of jaundice the liver cells were swollen and detached. Inflammatory infiltration followed. Patients dying on the seventh day showed mid-lobular necrosis. A case is reported of a child, aged 3½ months, who died on the eighth day of what was thought to be homologous serum jaundice following transfusion after birth for a suspected mild erythroblastosis. The liver showed diffuse subtotal necrosis and autolysis of liver cells infiltrating the stroma. *M. Le Vay.*

The Pathogenesis of Polycystic Livers. Reconstruction of Cystic Elements in Two Cases. NORRIS, R. F., and TYSON, R. M. (1947). *Amer. J. Path.*, **23**, 201.

This article describes the reconstruction of the cystic elements in portions of liver from 2 newborn infants. The livers had a normal contour, but microscopical examination revealed irregular cystic dilatations of the intrahepatic bile ducts, which were lined by cubical epithelium and encircled the branches of the hepatic artery and portal veins. The models, reconstructed from serial sections, show numerous anastomoses and irregular dilatations of the intrahepatic bile ducts, some of which were cut off into isolated segments. The authors consider that the lesions result from an abnormal extension of the normal processes of degeneration in the bile ducts, causing distension, segmentation, and cystic dilatation, while normal differentiation of the hepatic anlage occurs elsewhere. *E. T. Ruston.*

Liver Biopsy in Thyrotoxicosis. [In English.] PIPER, J., and POULSEN, E. (1947). *Acta med. scand.*, **127**, 439.

Liver-function tests were performed in 30 cases of thyrotoxicosis; liver biopsy was carried out in 15. In 20% of cases the Takata-Ara test was positive. An increase in the serum phosphatase of 11 or more Buch units (normal value 0 to 7 Buch units) occurred in 9 out of 28 patients. There was no significant alteration in the formol-gel test, prothrombin time, plasma colour, platelet count, serum iron and urine urobilin estimation. No relation between the metabolic rate and the results of liver-function tests could be demonstrated. The 15 liver biopsies showed normal liver tissue 5 cases, glycogen (picric-acid fixation) markedly reduced in 2 cases, slight steatosis in 3 cases, delicate dark streaks of cells in 5 cases, slight round-cell infiltration in 2 cases, and suspected commencing cirrhosis in 1 case. The authors point out that a single negative biopsy result cannot exclude the

possibility of more pronounced changes in the liver, but the peripheral zone is the most likely to show changes, and that is the zone examined in needle biopsy.

S. Oram.

Combined Anterior Pituitary Necrosis and Symmetrical Cortical Necrosis of the Kidneys following Accidental Haemorrhage. GRASBY, E. D. Y. (1947). *J. Obstet. Gynaec. Brit. Emp.*, **54**, 203.

Three cases of accidental uterine haemorrhage are described. In the first, necropsy revealed combined renal cortical necrosis and pituitary necrosis; in the second there was cortical necrosis with clinical and pathological evidence of less renal destruction than is usually seen; in the third there was probable recovery from cortical necrosis.

Epidemic Poliomyelitis. Some Pathologic Observations on Human Material. LUHAN, J. A. (1946). *Arch. Path.*, **42**, 245.

A detailed histological study of the central nervous system was made in 13 fatal cases from the 1943 Chicago epidemic of poliomyelitis. Duration of illness was 3 to 21 days, the mean being 6 days; clinically all cases showed evidence of bulbar involvement. Slight or moderate cerebral oedema was found in 12 cases. Blocks of tissue were examined histologically from eleven representative areas in the cerebral cortex; the typical changes were confined to the motor cortex in all but 1 case, this agreeing with findings of other workers. The olfactory bulbs and tracts were available for examination in only 5 cases; all were normal. Sections of medulla oblongata and cervical cord showed the inflammatory reaction to be more intense here than elsewhere; a table is given in which numerical estimates have been made of the intensity of the reaction in 14 different sites in the central nervous system. In 5 cases of 9 in which sufficient material was available to permit the comparison the most recent process (as evidenced by the large number of polymorphonuclear leucocytes present) was found in the lumbar, sacral, and lumbo-sacral segments. From a consideration of the clinical features and histological findings the author classifies his cases into 9 primary "bulbar" types, 3 primary spinal types, and 1 indeterminate.

W. S. Killpack.

Amyloid Macroglossia. Report of a Case. BABER, M. D. (1947). *Lancet*, **1**, 210.

A man of 63 had swelling and induration of his tongue causing difficulty in swallowing for about 6 weeks. He died from coronary thrombosis after an unsuccessful attempt had been made to insert an oesophagoscope. Necropsy revealed amyloid infiltration of the tongue, the buccal mucosa, the oesophagus, and the heart. The oesophagus was a thickened almost rigid tube from amyloid infiltration of its wall. The liver and spleen were normal, but there were a few deposits of amyloid substance in the glomerular tufts of the kidney. The condition differed from the classical form of amyloid disease which follows protracted suppuration, tuberculosis, and syphilis, and which commonly affects the liver and spleen.

J. B. Duguid.

Human Toxoplasmosis. A Clinicopathologic Study with Presentation of Five Cases and Review of the Literature. CALLAHAN, W. P., RUSSELL, W. O., and SMITH, M. G. (1946). *Medicine, Baltimore*, **25**, 343.

About 36 cases of human toxoplasmosis have been recorded, but there is reason to believe that the incidence is considerably higher and that it has a wide geographical distribution. This view is based on the detection of unsuspected infections in 60 out of 250 persons in the United States by means of the "neutralization test." The authors have critically examined and analysed the necropsy material and records of 10,000 cases in the department of pathology, Washington University School of Medicine, St. Louis. This search revealed 5 cases listed in the records under "chronic meningitis," "syphilitic meningitis," and "encephalomalacia with calcification." A clinico-pathological study of these cases, coupled with the finding of the causative organism in all of them, left no doubt that they represented undetected instances of toxoplasmosis.

Anuria Following Criminal Abortion. YOUNG, J., and WALKER, A. H. C. (1947). *J. Obstet. Gynaec. Brit. Emp.*, **54**, 196.

The authors describe a case of widespread uteroplacental injury followed rapidly by oliguria, azotaemia, and death. The patient, aged 31, single and nulliparous, was admitted after amenorrhoea lasting 11½ weeks. Nine hours before admission an abortionist had injected 12 oz. of "dettol," soap, and water into the uterus with an adapted Higginson's syringe. From the time of the injection to the patient's death (5¾ days) only 8¾ oz. of urine was excreted. There was no oedema. During that time the blood pressure rose from 90/75 to 140/90 mm. Hg, the blood urea from 115 mg. to 290 mg.% and the serum potassium from 23.6 mg. to 37 mg.%, while the plasma chloride fell from 540 mg. to 473 mg.%. The urine contained albumin and a normal concentration of urea.

At necropsy the placenta was found closely applied to the anterior wall of the uterus, but the membranes were separated from the posterior wall by gelatinous material containing fatty acids and smelling of dettol. This material reached to the upper edge of the placenta, the neighbouring 3 to 4 cm. of which was haemorrhagic and friable. The uterine wall in this area was pale, soft, and friable. The upper posterior uterine wall showed typical infarction, with vessels completely occluded with granular debris. The ovary contained a large corpus luteum, and there was enormous dilatation of veins and capillaries, with widespread interstitial haemorrhage. The foetus corresponded to 16 weeks of gestation. The classical picture of "transfusion" or "crushing injury" kidney was seen with pigmented debris in the tubules giving a positive benzidine reaction for haemoglobin. The pituitary showed a focus of necrosis in the pars anterior.

The authors consider that the soap and dettol injected under pressure probably entered the large veins of the uterus, and that much of the necrosis was the result of the direct action of the chemicals. The extensive haemorrhage was possibly due to the thrombosis of the main vascular channels. They point out the marked similarity of uterine and renal lesions in their case to those seen in concealed accidental haemorrhage. The renal lesions resemble those found after crushing injuries, extensive

burns, and "mismatched" blood transfusions, the common factor being the presence of extensive tissue damage. The blood transfusions could not be incriminated in this case, for the profound oliguria had begun before it was given. The anuria in this type of case is due to cortical ischaemia, and tubular blockage from blood casts and debris, with probably a direct toxic effect on the tubules. This cortical ischaemia has recently been ascribed to a short circuit of the total renal blood flow through the medullary vessels (Trueta *et al.*, *Lancet*, 1946, **2**, 237). Similar, but less severe, renal lesions, with pigment deposits, may be found in cases of eclampsia, and the authors consider them to be due to the massive placental damage found in such cases. *Aileen M. Dickins.*

A Cause of Rapid and Unforeseen Death of Infants : Milk Embolism in the Lung. (Sur une cause de mort rapide et imprévue du nourrisson: l'embolie de lait dans le poumon.) MARIE, J., SERINGE, P., and HÉBERT, S. (1947). *Sem. Hôp., Paris*, **23**, 1335.

The author thinks that in the 3 cases described the deaths of the infants were attributable to inhalation into the air passages of milk regurgitated from the stomach.

In each case the lungs showed histological evidence of patchy bronchopneumonia of very acute type, distinguished by the presence in bronchioles and alveoli in the affected areas of foreign material staining readily with fat stains. Such a histological picture could be exactly reproduced by the intratracheal injection into a guinea-pig of a few ml. of milk, the animal being killed 15 minutes after this procedure. Evidence of a vital reaction to the foreign material (compensatory emphysema, cellular reaction, and alveolar exudate) in the affected bronchopulmonary segments led the authors to conclude that the milk had found its way into the bronchial tree during life, and that its presence there was not due to post-mortem regurgitation. They suggest that this event is a not uncommon cause of sudden death in apparently healthy infants. In some cases reflex laryngeal spasm brought about by intratracheal inhalation may be sufficient to cause death from asphyxia. They advise, therefore, that babies under 2 months old should not lie horizontally in their cots, especially if they have a tendency to vomit, but should be propped semi-vertically with pillows. *M. MacGregor.*

Lesions of the Central Nervous System in Two Cases of Kernicterus. (Lésions du système nerveux central dans deux cas d'ictère nucléaire du nouveau-né.) BERTRAND, I. (1946). *Rev. hémat.*, **1**, 399.

The distribution of bile-stained areas and the histological changes found in the brains of 2 infants who died from kernicterus are described. The highest degree of bile staining was found in the amygdaloid nucleus, the thalamus, the hippocampal gyrus, the optic radiations, the depths of the occipito-parietal sulcus, the nuclei of the cranial nerves III to VIII inclusive and XII, all structures in the floor of the fourth ventricle, the olive, the dentate nucleus, especially the dorsal lamina, and the whole granular layer of the cerebellum. Less severe staining was found in the grey matter of the anterior perforated space, caudate and lenticular nuclei, body of Luys, superior corpora quadrigemina, and small areas on either side of the interparietal, superior frontal, and collateral sulci.

Histological changes were not confined to the bile-stained areas. They consisted of oedematous degeneration of the nerve cells affecting most of the cortex, with patches of more severe degeneration irregularly distributed but most marked in the bile-stained areas, and particularly affecting the dentate nucleus and the nuclei of the third and sixth nerves, which could hardly be identified because of the destruction of nerve cells. There was only slight glial reaction, with irregular perivascular infiltration of mild degree.

G. Discombe.

Multiple Myeloma. Review of Eighty-three Proved Cases. BAYRD, E. D., and HECK, F. J. (1947). *J. Amer. med. Ass.*, **133**, 147.

Cytochemical Differentiation between the Pentose and Desoxypentose Nucleic Acids in Tissue Sections. SAUNDERS, F. K. (1946). *Quart. J. micr. Sci.*, **87**, 203.

The intracellular distribution of the two kinds of nucleic acid is ascertained by comparing the enzyme-treated sections with their controls. Staining with celestine blue indicates the presence of desoxyribonucleic acid, and staining with pyronin that of the pentose acid. The results of the technique are illustrated by reference to the Purkinje cells of the guinea-pig. The specificity of the reactions is discussed, and it is concluded that the method provides a satisfactory means of distinguishing the two kinds of nucleic acid in fixed tissues.

A. K. Powell.

Cancer Cells in Prostatic Secretions. HERBUT, P. A., and LUBIN, E. N. (1947). *J. Urol.*, **57**, 542.

Urine obtained after digital massage of the prostate was examined for cancer cells. A diagnosis of carcinoma was made on cytological grounds in 17 of 100 cases. In 10 the diagnosis was confirmed histologically. In 7 it was not confirmed, but in 6 it was thought to be reasonably certain on clinical grounds. The authors think the results warrant further investigation.